Nederlandse Voedsel- en Warenautoriteit
Ministerie van Landbouw, Natuur en Voedselkwaliteit

> Return address PO Box 43006 3540 AA Utrecht

To the Minister of Agriculture, Nature and Food Quality and to the Minister for Medical Care and Sport

Office for Risk Assessment & Research

Catharijnesingel 59
3511 GG Utrecht
PO Box 43006
3540 AA Utrecht
www.nvwa.nl

Contact

T (0)88 223 3333
risicobeoordeling@nvwa.nl

Our reference
TRCVWA/2021/4183

Date
16 August 2021

Advice from the Director of the Office of Risk Assessment & Research on

Time-Temperature Indicators on Food Products

Background

One of the targets of the Ministry of Agriculture, Nature and Food Quality is to halve food waste by 2030 compared to the situation in 2015[1]. The Ministry's plans are in line with international developments, such as the European Commission's 2030 Agenda. One of the options that the Ministry of Agriculture, Nature and Food Quality is exploring is the use of a time-temperature indicator (TTI) on food products. A TTI is an instrument that measures the cumulative heat exposure of a food product on its packaging. Continuously measuring the temperature of the food from the point of packaging could make it possible to accurately determine the remaining sensory shelf life or safe consumption period for each individual product. This would be more reliable than a date shown on the label that applies to an entire batch. If the TTI indicates a shorter sensory shelf life or safe consumption period, it can improve food safety; if it indicates a longer sensory shelf life or safe consumption period, it can reduce food waste. TTIs are already used in some European countries, and the European Food Safety Authority (EFSA) has issued a positive recommendation for some variants.

To investigate whether TTIs can be used in the Netherlands to reduce food waste, an application was submitted by a Dutch supermarket chain. In cooperation with a university, this supermarket chain wants to carry out a practical trial of the 'Keep-it' TTI (Skjervold et al., 2007) for the perishable foods 'fresh chicken meat' and 'fresh fish or fresh fish fillet'. The supermarket chain and university anticipate that the use of the Keep-it indicator will help reduce food waste to some extent because they assume that these food products have a longer sensory shelf life or safe consumption period than the date stated on the label. If the results of the practical trial are positive, the supermarket chain plans to extend the use of the Keep-it indicator to its entire fresh fish and fresh chicken range. The practical trial involves food products with a use by date, which requires the Minister for Medical Care and Sport to grant a temporary exemption from Section 15(3) of the Preparation and Processing of Food (Commodities Act) Decree (Wbbl)[2]. To ensure food safety during the practical trial, the granting of such an exemption is subject to a food safety risk assessment. The Ministry of Health, Welfare and Sport has asked the Office for Risk Assessment & Research (BuRO) to carry out this risk assessment.

[1] https://zoek.officielebekendmakingen.nl/kst-31532-254.html
[2] Section 15, paragraph 3, of the Preparation and Handling of Food (Commodities Act) Decree (*Warenwetbesluit Bereiding en behandeling van levensmiddelen*):
3. Without prejudice to the applicable requirements regarding the labelling of food products, the packaging of the food or beverages referred to in paragraph 2(a) shall bear storage conditions stating, among other things, that the product must be consumed within a certain number of days after purchase, but never later than the pre-specified date.

Although permission was requested for a practical trial to include a limited number of food products that required heating prior to consumption and one specific time-temperature indicator, BuRO and the Ministry of Health, Welfare and Sport decided to issue an advisory report with a broader scope, namely whether the use of time-temperature indicators guarantees food safety in general.

The Ministry of Health, Welfare and Sport therefore asks BuRO to respond to the following questions:

1. What food safety risks are associated with the use of time-temperature indicators (TTIs) on food products?
2. If a practical trial were to be carried out on the use of TTIs on food products, what conditions would this project need to meet in order to manage the risks?

Approach

BuRO started with an interpretation of the different parts of the question posed. Both legal and scientific aspects were considered in order to identify the key aspects to consider on introducing the use of TTIs. This included the safety of the indicator as an instrument, its suitability as an indicator of food safety/fitness, expected conditions of use by the consumer and potential authorisation for wider use.

The four steps of the risk assessment were completed (Codex Alimentarius Commission, 2019) based on information obtained through a literature review (Appendix 1) and specific information on the Keep-it indicator provided by the Ministry of Health, Welfare and Sport. In addition, a survey was conducted among European countries to obtain information on their experience of TTIs in general and of Keep-it in particular (Appendix 2). At the time of this risk assessment, EFSA published a scientific opinion providing guidance on date marking and related food information such as storage instructions (EFSA, 2020). This opinion was incorporated in the final stage of the risk assessment. Since the TTI is also a form of date marking, albeit a flexible one, BuRO provides an indication as to where the TTI could fit in to this phased plan. The draft version has been reviewed by external experts.

Scope and definition

TTIs have very different working mechanisms. In this risk assessment, we focus on TTIs that are separated from the food product and from the consumer by a functional barrier.

As use by and best before dates are a legal requirement, this risk assessment focuses on the use of the TTI in addition to the use by/best before date.

A TTI is affixed to and activated on the food packaging during the production process. For the pre-packaging stage, food safety in relation to TTIs is similar to that of the printed best before date. The process up to packaging is not part of this risk assessment. However, the varying levels of initial contamination of a packaged food product have been taken into account as different starting points and subsequent risks.

Although the question posed by the Ministry of Health, Welfare and Sport focuses on food safety, organoleptic quality is also a relevant factor when assigning a printed date. Insofar as this quality is related to temperature, BuRO has included it in this assessment.

The use by date is an indication enshrined in law after which a food product is considered unsafe. The focus in Dutch legislation lies on cooling and storage time. This risk assessment has been drawn up in the knowledge that Section 15 paragraph two[3] of the Preparation and handling of Food (Commodities Act) Decree is to be amended, whereby the focus will be on the period within which the product is safe to eat, whether or not limited by the growth of pathogens (particularly *Listeria monocytogenes*). The risk assessment therefore takes this into account. This approach is in line with the recently published EFSA scientific opinion on date marking(EFSA, 2020).

Findings

- Time-temperature indicators can be based on an enzymatic, chemical or microbiological principle.
- In view of international developments and the national objectives to reduce food waste, it is worthwhile exploring the potential use of the TTI in the Netherlands through a practical trial. In order to guarantee food safety during such a trial, BuRO is of the opinion that the following steps are important, based on EFSA opinions (EFSA, 2013;2016a;2020):

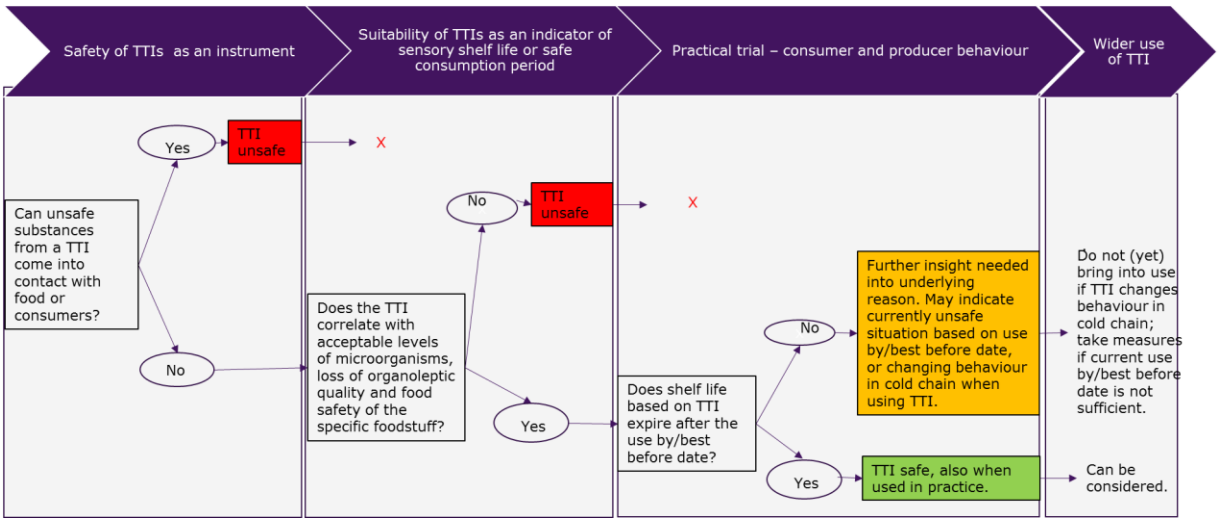

Figure 1: Stepwise approach to assessing food safety associated with a TTI.

- Keep-it is a TTI that works according to a chemical principle and has a functional barrier that is not breached under normal circumstances. The active chemical substances in the TTI are not released at a wide temperature range (-30˚C to 90˚C). At a constant temperature of 4˚C, colour development increases to 50% after 19 days, and to 93% of the indicator after 76 days, making it suitable for use with refrigerated and possibly also non-refrigerated food products. BuRO does not know

[3] Section 15, paragraph 2, of the Preparation and Handling of Food (Commodities Act) Decree:

2. If the packaging of a food or beverage product referred to in paragraph one features:
a. storage conditions stating that the product must be stored at between 0°C and 6°C; or
b. a best before date that lies less than 5 days ahead;
Article 24(1) of Regulation (EU) 1169/2011 on the use by date shall apply.

whether Keep-it has submitted a dossier in accordance with the EFSA guidelines for authorisation of the TTI. Enquiries with Member States have not yielded this information.

- Safety during normal use is sufficiently guaranteed where incorporated by the company in an adequate HACCP system[4].
- It is not possible to estimate the chemical risks in the event of breakage of the indicator due to a lack of data on exposure. This specifically concerns the concentration of the substances present and the absolute quantity in the indicator that could potentially be released in the event of an incident.
- Chemical substances used in a TTI must be legally approved under Regulation (EC) 450/2009.
- The correlation of the indicator with the shelf life of the proposed food products (chicken drumsticks, tilapia fillets) was tested on the basis of aerobic plate count, *Enterobacteriaceae* and *Pseudomonas* spp. In 6 out of 16 temperature profiles tested, Keep-it incorrectly indicates a longer storage period, which can have a negative effect on food safety. The correlation of Keep-it with histamine formation was not tested. Keep-it will not be used for ready-to-eat food at this time.
- Research shows that you cannot just assume safe use of a TTI and anticipate a reduction in the amount of safe food thrown away compared to wastage based on use by or best before dates. This is because the accuracy, and therefore the safety, of the indication may differ between food products under the influence of the specific microbiological properties of a food product and the recipe, due to other factors such as light and moisture. The pathogenic microorganisms listed in the table below are relevant when testing the TTI in relation to date marking of perishable foods.
- The importance of calibrating a TTI to the food product has already been demonstrated by research carried out by the Netherlands Organisation for Applied Scientific Research (TNO) several decades ago, in which one of the three TTIs tested expired when the food product was still of a satisfactory quality, which would actually encourage food waste.
- The probability of unsafe food is low if the necessary date marking studies have been carried out for the TTI-food combination, with TTI being assessed as a flexible date, and moderate if such studies have not been carried out. The probability of unsafe food becomes lower if the TTI is affixed at the start of the production phase for the packaged food product, i.e. at the time the necessary studies are carried out, and higher if the TTI is affixed by a food business operator at a later stage, because it is more likely that the necessary studies may not be carried out at that time, or the operator will not have sufficient knowledge of the microorganisms relevant for the product. The risk of the TTI indicating a sensory shelf life or safe consumption period that is too long is further increased where changes are made to recipes earlier in the chain and such changes are not communicated to later stages of the chain.
- Finally, consumer behaviour is also important, in terms of whether they understand what the TTI aims to do in combination with, or instead of, the current date marking.

[4]Hazard Analysis and Critical Control Points

- In addition, the TTI itself must be sufficiently safe as an instrument to be used with food products and must not contain any unsafe substances.
- It may also transpire that the TTI indicates a shorter sensory shelf life or safe consumption period, which can help to improve food safety on the one hand, but could on the other hand also be the result of changing behaviour in the cold chain leading to potential food safety risks.

Table 1: Relevant pathogenic microorganisms in relation to date marking of perishable foods.

Hazard	Relevant according to BuRO	Relevant according to EFSA	Legal criteria	Foodstuffs (several examples)
Growth required during safe consumption period				
Histamine	Yes	No	Yes	Fish
Bacillus cereus	Yes	Yes	Yes	Pre-cooked or heated rice, pasta, potatoes; pasteurised milk products.
Yersinia enterocolitica	Yes	Yes	No	Meat and meat products
Listeria monocytogenes	Yes	Yes	Yes	Ready-to-eat products, salads, smoked fish, soft cheeses
Staphylococcus aureus	Yes	Yes	Yes	Meat, fish, cheese, dairy products, prepared meals
Vibrio spp.	Yes	No	No	Fish, seafood and shellfish
No growth required during safe consumption period				
Clostridium botulinum	Yes	Yes	No	Canned fish, pâté, hummus
Salmonella	Yes	Yes	Yes	Meat, fish, vegetables, fruit, dairy products, prepared salads
STEC	Yes	Yes	Yes	Meat, fish, vegetables, fruit, dairy products, prepared salads
Shigella spp.	Yes	No	No	Salads, chicken, dairy products, tuna, raw vegetables

Answer to the question

1. *What food safety risks are associated with the use of time-temperature indicators (TTIs) on food products?*

With regard to the safety of the indicator as an instrument:

- The risk that the TTI may contain unsafe active substances that may come into contact with food or the consumer if the barrier between the TTI and the food product and the consumer is not adequate or is breached;
- The risk that packaging bearing TTIs that contain chemical substances not on the positive list is recycled into food contact materials;

With regard to accurate indication of the safety of the food product:

- If the progression and expiry of the TTI are not properly calibrated to the specific properties of the proposed food product (a_w, pH, spore elimination, subsequent contamination, bactericidal post-treatment), there is a risk that spoilage organisms or pathogenic microorganisms relevant to the food product (particularly *L. monocytogenes, Yersinia spp., Proteus* [histamine] and to a lesser extent *Vibrio spp., S. aureus, B. cereus*) may grow to pathogenic levels. This is more likely to happen if the TTI has a default expiry time, and if the TTI is affixed to the food product at a later stage in the chain;
- If the producer of the specific food product has not carried out sufficiently substantiated studies and does not take responsibility for safety during the sensory shelf life or safe consumption period indicated by the TTI, there is a risk that the TTI may incorrectly indicate that the product is safe;
- The risk that the TTI will become unreliable in the event of untested, but expected, temperature profiles, such as a temperature shock just before a best before date or a use by date, longer time outside the refrigerator, higher refrigerator temperature at the consumer (i.e. between 3.8 and 11.6 °C);
- The risk that if consumers or food business operators fail to follow the storage recommendations and instructions (storage temperature, heating prior to consumption, use after defrosting), the TTI will incorrectly indicate that the product is safe. Although this also applies to use by/best before dates, it is not known whether consumer or food business operator behaviour changes as a result of confidence in the performance of the TTI;
- The risk that if the progression and expiry of the TTI are not adjusted in the event of recipe changes that affect shelf life, such as less sugar, less salt, less preservatives, the TTI will incorrectly indicate a longer shelf life.

2. *If a pilot were to be carried out on the use of TTIs on food products, what conditions would this pilot need to meet in order to manage the risks?*

a) Safety of TTIs as an instrument

- the TTI must be authorised as a food contact material in accordance with the European Regulation (450/2009);
- it must have been demonstrated that the TTI remains safe in extreme, but realistically expected, temperatures such as freezing temperatures and heat waves;
- it must be stated whether the substances are on the positive list for plastic food contact materials as set out in Regulation (EU) 10/2011; and if they do not feature on the positive list, measures must be taken to prevent the TTI from being recycled into plastic food contact materials.

b) Food safety

For every TTI-food product combination:

- The party affixing the TTI to the food product must be held responsible for its safety during the safe consumption period indicated by the TTI, by referring that party to the microbiological criteria applicable to the food product in question (Regulation [EU] 2073/2005 Article 3; Info Sheet 85) and acceptable levels of any other pathogenic microorganisms relevant to the specific food product, whereby the progression of the TTI must reflect the dynamics of these microorganisms. To this end, the above table and the decision tree drawn up by EFSA (Appendix 3a) and the additions made to the

decision tree by BuRO specifically in relation to TTIs (Appendix 3b, and points I to IV in the supporting information), with the corresponding underlying studies, can be followed;
- 'Accidental breach of the TTI barrier' from the time of its application in the production process must be included in the HACCP;
- The HACCP must also state that in the event of recipe changes that affect shelf life (such as less salt or less sugar), the TTI must be recalibrated to the specific properties of the food product.

If the above points in relation to TTI-food product combination are found to be sufficient, additional conditions for the practical trial:
- A temporary exemption must be granted from Section 15(3) of the Wbbl.
- It must be assessed whether the use of a TTI is temporarily legally possible, for example, if the TTI expires after the use by date and the food product is therefore no longer considered safe by law, or if different expiry dates could lead to confusion among consumers (European Labelling Regulation).
- The practical trial must be used to evaluate the following aspects:
  - behavioural change in the cold chain on the part of consumers or food business operators, involving temperatures that have not been tested as expected conditions;
  - consumer understanding of TTI use in addition to use by/best before dates;
  - whether a TTI identifies a current unsafe situation if the TTI indicates a *shorter* safe consumption period than the use by date.

Office for Risk Assessment & Research

Date
16 August 2021

Our reference
TRCVWA/2021/4183

Advice

To the Minister for Medical Care and Sport

- Impose conditions on a TTI manufacturer and a food business operator that affixes the TTI if they wish to carry out a practical trial to ensure safety of use on food products by taking into account the factors mentioned under question 2. Use EFSA's science-based date marking decision tree in this process, and include TTI as a flexible form of date marking, as shown in Appendix 3b. As a further condition of the trial, require the specific identification of the TTI and the recording of the point in the production process when the TTI is affixed to the packaging.
- Commission research, for example in conjunction with the practical trial of the TTI, into how consumers perceive instructions on TTI packaging, and whether this raises the possibility of behavioural change by consumers and food business operators.
- Consider, and commission research into, whether the use of a TTI on other food products can be supported after the practical trial referred to above.
- Follow the regular statutory authorisation procedure for TTIs as 'active or intelligent substances present in active and intelligent materials and articles intended to come into contact with food' according to the European Regulation (EC 450/2009). Ensure that the use of the TTI alongside or instead of the use by and/or best before date is enshrined in law.

- In anticipation of the possible safe use of this TTI, initiate the associated legal safeguards. Require the operator to follow the same steps for each new TTI-food product combination.

Yours sincerely,

Prof. A. Opperhuizen
Director of the Office for Risk Assessment and Research

Table of contents

Substantiation

What is: unsafe, injurious to health, or unfit in relation to food products?
According to the law, a food product must not be unsafe, as stated in Regulation (EC) No. 178/2002, Article 14, paragraph 2:

a) injurious to health; or
b) unfit for human consumption.

Unsafe in the sense of injurious to health means that the food may have an adverse effect on health when consumed. For example, if pathogens are present in the food that exceed normal levels. Or if pathogen growth is required in order to cause disease, and this degree of growth is possible during the storage period.

Unsafe in the sense of unfit is defined as follows in the guidance to Regulation (EC) No. 178/2002(EU, 2010):

"Unacceptability is a central factor in assessing whether a food is unfit for human consumption. A foodstuff may become unfit for consumption as a result of contamination, for example a high contamination with non-pathogenic microorganisms (see Article 14[3] and [5] of the Regulation), the presence of foreign material, an unacceptable taste or smell or an even more evident deterioration in quality such as putrefaction or decay." This means that if a food is unfit, it does not make the consumer sick, however the food is still unsafe. Consumers will generally notice if a food product is unfit and choose not to consume any or all of the food product.

'Harmful' food products constitute a food safety risk; 'unfit' food products concern a loss of organoleptic quality.

What is: a best before date or a use by date?

A best before date or use by date is determined by the food business operator and stated on the product packaging. This date is determined under conditions that are consistent with the distribution, storage, sale and use of the food product as expected to take place, whereby the food remains safe, i.e. *not* becoming *harmful* or *unfit*.

The European Labelling Regulation (No. 1169/2011) states the following:

> Article 24 Minimum durability date, 'use by' date and date of freezing:
> 1. In the case of foods which, from a microbiological point of view, are highly perishable and are therefore likely after a short period to constitute an immediate danger to human health, the date of minimum durability [note: corresponds to 'best before' date] shall be replaced by the 'use by' date. After the use by date a food shall be deemed to be unsafe in accordance with Article 14(2) to (5) of Regulation (EC) No. 178/2002.

This means that for a food in which microorganisms can grow after a short period of time, and the safe consumption period is limited by growth up to the legal limit, a use by date must be used because the food may be harmful after that time. However, if it can be demonstrated that no growth of harmful microorganisms takes place in a food (e.g. very dry foods, extremes in pH and adequately preserved foods) at the recommended storage temperature, a use by date is not mandatory and a best before date may be used. After a best before date, the food may become unsafe due to unfitness, however the food is not harmful according to the legal definition.

In Dutch legislation, the static expiry date is incorporated in a different way, namely with a focus on refrigeration and time. The Preparation and Handling of Food (Commodities Act) Decree (WBBL) states:

> " 1 Food or beverages or raw materials that must be kept chilled in order to prevent microbiological spoilage or the growth of pathogenic bacteria must:
> in the case of pre-packaged food or beverages or raw materials, be transported or stored in such a way that the temperature of the goods does not exceed the temperature indicated by the manufacturer; or,
> insofar as the manufacturer does not indicate a specific storage temperature on the pre-packaging or the goods are not pre-packaged, be transported or stored in such a way that the temperature of the goods does not exceed 7°C.
> 2 If the packaging of a food or beverage referred to in paragraph one:
> features storage instructions stating that the goods must be kept between 0°C and 6°C; or
> states a shelf life of less than 5 days;
> Article 24(1) of Regulation (EU) 1169/2011 on the use by date shall apply."

According to Dutch legislation, food that is kept at >6°C and/or has a shelf life of at least 5 days can therefore bear a best before date while the shelf life is limited by the growth of microorganisms up to the legal limit. As this is not in line with the EU legislative text, the WBBL is to be brought in line with European legislation.

In terms of food safety, the safe period is therefore related to harmfulness due to pathogen growth. These are foods that bear a use by date according to Dutch legislation, or should bear a use by date according to European legislation, which is the latest date on which it is still safe (i.e. not harmful) to consume the food. Pathogenic microorganism growth is usually not noticeable to the consumer, which makes this date particularly important in ensuring food safety.

In terms of loss of organoleptic quality, the safe period is related to unfitness due to other factors such as spoilage organisms, dehydration, chemical or enzymatic changes. Since loss of organoleptic quality can be noticeable to the consumer, it is unlikely that they will consume any or all of the food. The food may still be acceptable to the consumer after the best before date. For this reason, the best before date is not that important in terms of food safety.

When pathogenic microorganism growth is possible, but is limited or delayed by preservation to such an extent that loss of organoleptic quality and unfitness always occur before harmfulness, the use of a best before date is acceptable from a food safety point of view.

What is: a shelf-life study?
Food business operators must ensure that foodstuffs comply with microbiological criteria during the sensory shelf life or safe consumption period. This obligation is set out in Article 3 of Regulation (EU) 2073/2005 on microbiological criteria for foodstuffs (EC, 2008). This includes studies to investigate compliance with these microbiological criteria throughout the shelf life (paragraph 2):

> Article 3 of Regulation (EU) No 2073/2005:
> 1. Food business operators shall ensure that foodstuffs comply with the relevant microbiological criteria set out in Annex I. To this end the food business operators at each stage of food production, processing and distribution, including retail, shall take measures, as part of their procedures based on HACCP principles together with the implementation of good hygiene practice, to ensure the following:
>
> a) that the supply, handling and processing of raw materials and foodstuffs under their control are carried out in such a way that the process hygiene criteria are met,

b) that the food safety criteria applicable throughout the shelf-life of the products can be met under reasonably foreseeable conditions of distribution, storage and use.

2. As necessary, the food business operators responsible for the manufacture of the product shall conduct studies in accordance with Annex II in order to investigate compliance with the criteria throughout the shelf-life. In particular, this applies to ready-to-eat foods that are able to support the growth of *Listeria monocytogenes* and that may pose a *Listeria monocytogenes* risk for public health.

Food businesses may collaborate in conducting those studies.

Guidelines for conducting those studies may be included in the guides to good practice referred to in Article 7 of Regulation (EC) No 852/2004.

Regulation (EU) 2073/2005 *ANNEX II:*
The studies referred to in Article 3(2) shall include:

— specifications for physico-chemical characteristics of the product, such as pH, aw, salt content, concentration of preservatives and the type of packaging system, taking into account the storage and processing conditions, the possibilities for contamination and the foreseen shelf-life, and

— consultation of available scientific literature and research data regarding the growth and survival characteristics of the micro-organisms of concern

When necessary on the basis of the above-mentioned studies, the food business operator shall conduct additional studies, which may include:

— predictive mathematical modelling established for the food in question, using critical growth or survival factors for the micro-organisms of concern in the product,

— tests to investigate the ability of the appropriately inoculated micro-organism of concern to grow or survive in the product under different reasonably foreseeable storage conditions,

— studies to evaluate the growth or survival of the micro-organisms of concern that may be present in the product during the shelf-life under reasonably foreseeable conditions of distribution, storage and use.

The above mentioned studies shall take into account the inherent variability linked to the product, the micro-organisms in question and the processing and storage conditions.

What is: a TTI?

The indicator as an instrument
TTI stands for time-temperature indicator. A TTI is an instrument that measures the cumulative amount of heat to which an individual product has been exposed, whereby the lower the temperature, the slower the progression of the gauge. The indicator is generally affixed to the packaging. A TTI can work on the basis of a chemical, enzymatic, mechanical, electrochemical or microbiological reaction with a visible indication of the outcome, for instance in the form of a colour change (Brody, 2001; Sohail et al., 2018). For TTIs, these different working mechanisms are described as follows (Realini & Marcos, 2014; Koutsoumanis & Gougouli, 2015):

- Mechanism based on an oxidisable chemical system that is regulated by temperature-dependent diffusion, whereby a colour change occurs in the

indicator label through reaction with a film (3M MonitorMark, Monitor MarkTM, Freeze WatchTM).
- Mechanism based on an enzymatic principle, whereby a colour change is induced by a change in pH which, following activation, is the result of controlled enzymatic hydrolysis of a lipid substrate (Check Point®, VITSAB®). The hydrolysis of the substrate causes the release of acid and therefore a lower pH, which is translated into a colour change.
- Mechanism based on solid-state polymerisation. These TTIs change from the point of production and it is therefore important that they are stored deep frozen, so that the change prior to use is minimal (Temptime Freshness Monitor®, Fresh-Check®).
- Mechanism based on a microbiological principle. These TTIs use patented strains of bacteria, whereby growth and metabolic activity produce visible changes in the indicator. The growth of bacteria causes a change in pH in the medium. These TTIs should also be kept frozen to minimise growth prior to use ((e0) ®, TRACE0 ®).
- Mechanism based on photosynthesis activated by UV light (e.g. OnVuTM). Once activated with UV light, the 'smart' ink reverts to colourless depending on time and temperature.
- Mechanism based on barcode labels whereby ink fades under the influence of temperature (FreshCode®, Tempix®).
- Mechanism based on a chemical reaction that is dependent on temperature and time, with a mobile and immobile component in separate compartments, which come into contact with each other following activation (Keep-it®). After activation, the mobile component progresses on the basis of a calibrated time-temperature combination towards the immobile component, causing a colour change.

Use of TTIs on food products
A number of regulations apply to TTIs as instruments for use on food products. Regulation (EU) 10/2011 sets out a positive list of authorised components of plastic food contact materials. Regulation (EC) 450/2009 sets out specific requirements for the commercialisation of active and intelligent materials and articles. Only substances included in the Union list of authorised substances, or that are authorised as food additives, or that are separated by a functional barrier may be used in components of active and intelligent materials and articles, provided they do not fall into the category of 'mutagenic', 'carcinogenic' or 'toxic for reproduction'. Authorisation takes place via an established procedure[5]. To apply for authorisation, the manufacturing sector must submit a dossier to the national competent authority (in the Netherlands the Ministry of Health, Welfare and Sport), which will forward this dossier to EFSA. The dossier must be submitted in accordance with the applicable guidelines (EFSA, 2009). EFSA evaluates the dossiers submitted and issues an opinion on authorisation. For the purpose of Dutch legislation, the G4[6] evaluates the dossier and advises the Ministry of Health, Welfare and Sport on the authorisation of the substance and any restrictions.

In principle, the active substances of TTIs do not come into contact with food. TTIs qualify as intelligent packaging incorporating a barrier. Regulation 450/2009 contains the following provisions in this regard:

Regulation (EU) No 450/2009 on active and intelligent packaging

[5] https://ec.europa.eu/food/safety/chemical_safety/food_contact_materials/authorisations_en
[6]The G4 committee of the National Institute for Public Health and the Environment (RIVM) assesses the risks associated with chemical substances proposed by companies for use in food packaging materials, as provided for in the Commodities Act (Packagings and Consumer Articles) Regulation.

Article 5(2)(c):
(c) substances used in components that are not in direct contact with food or the environment surrounding the food and are separated from the food by a functional barrier provided that they comply with the conditions set out in Article 10 and that they do not fall within either of the following categories:
(i) substances classified as 'mutagenic', 'carcinogenic', or 'toxic to reproduction' in accordance with the criteria set out in sections 3.5, 3.6 and 3.7 of Annex I to Regulation (EC) No 1272/2008 of the European Parliament and of the Council (1); NL L 135/6 Official Journal of the European Union 30.5.2009 (1) OJ L 353 of 31.12.2008, p. 1.
(ii) substances deliberately engineered to particle sizes so small that they exhibit functional physical and chemical properties that significantly differ from those at a larger scale.
Article 10:
Substances referred to in Article 5(2)(c)
1. The migration into food of the substances from components that are not in direct contact with food or the environment surrounding the food, as referred to in Article 5(2)(c) of this Regulation, shall not exceed 0.01 mg/kg, measured with statistical certainty by a method of analysis in accordance with Article 11 of Regulation (EC) No 882/2004 of the European Parliament and of the Council (1).
2. The limit provided for in paragraph 1 shall always be expressed as a concentration in foods. It shall apply to a group of substances, if they are structurally and toxicologically related, in particular isomers or substances with the same relevant functional group, and shall include possible set-off transfer.

A TTI is an instrument that is affixed to the packaging of a food product on a voluntary basis. The TTI may therefore legally fall under 'voluntary food information', as it provides information on the shelf life of the food. The Labelling Regulation states the following with regard to voluntary food information and information on shelf life:

Chapter V, Article 36 of Regulation (EU) No 1169/2011 Labelling Regulation:
1. Where food information referred to in Articles 9 and 10 is provided on a voluntary basis, such information shall comply with the requirements laid down in Sections 2 and 3 of Chapter IV.
2. Food information provided on a voluntary basis shall meet the following requirements:
a) it shall not mislead the consumer, as referred to in Article 7;
b) it shall not be ambiguous or confusing for the consumer; and
c) it shall, where appropriate, be based on the relevant scientific data.

Chapter III, Article 7(1)(a): Fair information practices
1. Food information shall not be misleading, particularly:
a) as to the characteristics of the food and, in particular, as to its nature, identity, properties, composition, quantity, durability, country of origin or place of provenance, method of manufacture or production;

BuRO's interpretation of aspects relevant to the use of TTIs
As use by and best before dates are a legal requirement, and the European legislation in this regard will not change in the near future, this risk assessment focuses on the use of TTIs in addition to use by/best before dates. However, if this risk assessment works out in favour of the use of TTIs, the potential use of a TTI as a replacement for use by/best before dates will be anticipated where possible.

The purpose of this approach is to keep up with international developments aimed at reducing food waste.

Safety of the instrument when used on food products
An instrument affixed to food must not contain substances classified as 'mutagenic', 'carcinogenic' or 'toxic for reproduction'. The absence of such substances must therefore have been clearly established before a TTI can be used on a food product.
BuRO notes that it is debatable whether a TTI should be considered 'voluntary food information' as referred to in the Labelling Regulation, which requires that this information must not be ambiguous or confusing for the consumer. BuRO is of the opinion that, if the TTI does not fall under this legal definition, it is still undesirable from a food safety point of view for the information provided by the TTI to be confusing or misleading for consumers.

Aspects relevant to the use of TTIs with a view to food safety
Pathogenic microorganisms are the most important factor when it comes to food safety in terms of safe consumption period and, as stated in the law, can lead to pathogenic food within 'a short period of time'. There is some debate as to how long this 'short period' can be, as EFSA also points out (EFSA, 2020). Date marking and storage instructions with a view to food safety are therefore mainly aimed at preventing the growth of microorganisms (to a pathogenic level). Temperature control in relation to the ambient temperature, i.e. refrigeration or adequate freezing, is one such measure that limits or stops the growth of microorganisms. In the case of deep-frozen foodstuffs, there is no increased microbiological risk provided the freezing chain is maintained. BuRO is therefore of the opinion that a *TTI has added value for both chilled and frozen foods*, as time and temperature are the main factors determining the sensory shelf life or safe consumption period. In the case of chilled foods, it is important that the food remains safe until the TTI period has expired; in the case of frozen foods, the added value of a TTI is that it can monitor whether a freezing chain has been interrupted, which would have given pathogenic microorganisms an opportunity to grow. Where a deviation from the storage temperature established in the study occurs, the use by or best before date may not be correct. In these cases, a TTI can provide consumers with more accurate information (Hogan & Kerry, 2008; Sohail et al., 2018).

Aspects relevant to the use of TTIs with a view to loss of organoleptic quality
In addition to food safety, the organoleptic quality (or 'fitness' as used in the law) of a foodstuff is also important, and is subject to temperature influences and therefore relevant in relation to the use of a TTI. Here too, when foodstuffs need to be kept cool in order to preserve their organoleptic quality, the most important factor is microorganisms. And in this case, it is not the pathogenic microorganisms, but *the spoilage microorganisms*. Of course, both pathogenic and spoilage microorganisms can be present in food at the same time. However, as also described by EFSA (EFSA, 2020), pathogenic microorganisms will not generally dominate in number unless the food has been treated in such a way that the background microbiota is drastically reduced. Where organoleptic quality is the limiting factor for the consumption period, this means that the spoilage microorganisms grow faster, causing spoilage that is noticed by the consumer and which results in all or part of the food not being consumed. *For this reason, loss of organoleptic quality due to spoilage microorganisms is also relevant in relation to a TTI.*
In addition to microbiological processes, chemical and, to a lesser extent, physical processes can also lead to a pathogenic foodstuff or to a loss of organoleptic quality. However, these processes take longer than microbiological processes, or

need repeated exposure to be pathogenic. *In relation to safety or fitness for use of a TTI, BuRO therefore does not consider chemical and physical forms of decay to be relevant*. However, these processes are also partly influenced by temperature. This means that chemical processes are also inhibited as an additional benefit of cooling or freezing. Unlike microbiological processes, chemical processes continue in a frozen state. The shelf life of frozen foods is generally limited by chemical spoilage, which is not directly pathogenic. This means that in frozen foods, chemical hazards may after a long time become the most relevant factor in terms of quality loss. In exceptional cases, after a long period of time or after repeated exposure, a chemical food safety hazard can also occur. However, these are problems that cannot be prevented by either a TTI or a best before date as illustrated by the following practical example:

> In the past, the Netherlands Food and Consumer Product Safety Authority (NVWA) had to deal with a case in which meat products had been stored packaged without a date for a long period of time. This period was estimated to be 15 to 20 years. As soon as they came on the market, and were therefore available to consumers, a date would be assigned to them. However, the NVWA found it likely that the food would no longer be acceptable for consumption. Proving this turned out to be complicated. The aerobic plate count criterion showed no microbiological spoilage. This corresponds to the frozen state of the food. Although there were objective parameters to determine certain forms of chemical or physical decay, these had not been transposed into enforceable standards. In order to identify chemical or physical decay, an organoleptic panel was called in to assess whether the defrosted food would be acceptable for consumption (personal communication of Olaf Stenvers).

Legal criteria
There are no legal criteria for the identification of chemical or physical decay, however there are indeed legal criteria for pathogenic microorganisms and spoilage organisms. These cover the most common microbiological hazards affecting food safety or fitness. Nevertheless, every foodstuff has its own characteristics and its own relevant microorganisms. Therefore, the *assessment of relevant microorganisms should be made on a case-by-case basis*. This means that microorganisms for which no criteria exist may also be relevant for a particular foodstuff. It is the responsibility of the food business operator to also ensure safety and fitness under the influence of these additional relevant microorganisms. The summary tables (tables 1 and 2) presented by EFSA for use by/best before dates (EFSA, 2020) can also be used for TTIs.

Conflict between legislation and practice
The legislation contains areas of conflict that can lead to debate regarding the use of a use by or best before date, and consequently the safe use of a TTI. It can generally be said that use by dates are aimed at food safety, and best before dates at organoleptic quality. According to the law, loss of organoleptic quality, or spoilage, is by definition not pathogenic. On the one hand, because it is not a hazard, and on the other, because the food has become unattractive to the consumer and will therefore not be consumed in its entirety. However, although the legislation focuses on safety (and therefore the use by date) for a 'short period of time', this does not mean that a foodstuff cannot become pathogenic after a longer period of time. *Listeria monocytogenes* is an example where, even if the product bears a best before date, it can still be pathogenic before the loss of organoleptic quality is such that the food is no longer attractive for consumption. Another possibility where spoilage can still be harmful is when consumers are in the habit of cutting out the mouldy part of a foodstuff and consuming the

remainder (throughout their lives). This can lead to accumulation, which can increase the risk of cancer (for example due to aflatoxins and mycotoxins).

Use of TTIs on food products with a long shelf life
TTIs can potentially indicate a wide time period and temperature range. They can therefore be used as shelf life indicators for both long-life best before date foods and for highly perishable foods with a use by date. Whereas for frozen or chilled food, temperature is the most important factor determining the sensory shelf life or safe consumption period, for long-life food, temperature is less important. These foods can be stored at ambient temperature. However, when stored for a very long time, or under unfavourable conditions, chemical or physical decay can occur, with temperature being only one of the relevant aspects. TTIs only cover the influence of temperature, and in many cases not a period of years. The literature shows that TTIs are mainly used on food products to monitor the cold chain of a foodstuff. Vaikousi et al. tested a microbiological indicator based on metabolic activity of *Lactobacillus sakei* in a range from 0 to 16°C. Tipping points triggering the TTI to indicate the end of shelf life occurred between 27 days (when stored at 0°C) and 2.5 days (when stored at 16°C) (Vaikousi et al., 2008). Anbukarasu et al. calculated for a TTI based on an enzymatic principle that 6 hours at 37°C to 7 days at 4°C TTI is considered optimal for foodstuffs that need to be stored at 4°C (Anbukarasu et al., 2017). Choi et al. tested a timer ('self-healing nanofiber-based mat') that can be set from 0.5 hours to 22.5 hours and can monitor both freezing to -20°C and cooling to 2°C (Choi et al., 2020). The review by Strother et al. (Sohail et al., 2018) provides an overview of a number of commercially available TTIs (OnvuTM, Fresh-Check®, (e0) ®, TRACE0®, Monitor MarkTM, Freeze WatchTM, Check Point®), which also focus on cold chain monitoring. *In BuRO's opinion, TTIs are therefore of limited added value for long-life foods, as a TTI only indicates the temperature in relation to time, and the specified best before date takes more aspects into account for the shelf life and storage conditions*.

Expected conditions of use by the consumer
Although the legislation distinguishes between foods on the basis of whether or not they need to be heated prior to consumption, EFSA states (EFSA, 2020) that this is in fact irrelevant when choosing a best before and a use by date, because cross-contamination can occur. A consumer may also consume the food insufficiently heated, contrary to the instructions. BuRO shares this view, also in relation to introducing the use of a TTI. *The main issue in relation to the sensory shelf life or safe consumption period is whether foodstuffs have undergone treatment to eliminate microorganisms or whether they have properties that prevent microorganisms from developing or forming toxins.*

Actipact fair project
To facilitate the use of active and intelligent food packaging systems in Europe and to improve food quality and safety, a research programme was set up between 1999 and 2001 with the aim of issuing recommendations for European directives (TNO, 2002). The NVWA was also involved at the time. The findings for TTIs were reported in an internal report as follows:

> Of the three time-temperature indicators tested, one was found to expire too early, namely at a time the foodstuff (smoked trout) was still of adequate quality. This was an indicator with a preset default expiry time of 19 days at 3°C. The conclusion reached was that TTIs can only provide indirect information on the quality of a food and should therefore only be considered as *additional* information, with no claims that they reflect product quality.

Specific information on Keep-it
The trial that the supermarket chain plans to carry out in cooperation with the university will be conducted with the 'Keep-it' TTI. This particular TTI will therefore be discussed in greater detail. Although the patent for Keep-it indicates that this TTI can be used between -30°C and 90°C, Keep-it is also mainly used to monitor the cold chain of a food product (Koutsoumanis & Gougouli, 2015). The information supplied by the Ministry of Health, Welfare and Sport shows that Keep-it has been tested for a number of foreseeable conditions, such as 2 hours at 25.3°C as a simulation of shopping by the consumer, and up to 13 days at 2.1°C as an optimal situation (Bouwman et al., 2019).

Risk assessment

Based on EFSA opinions (EFSA, 2013;2016a;2020) and BuRO's interpretation, the question posed by the Ministry of Health, Welfare and Sport is divided into several parts:

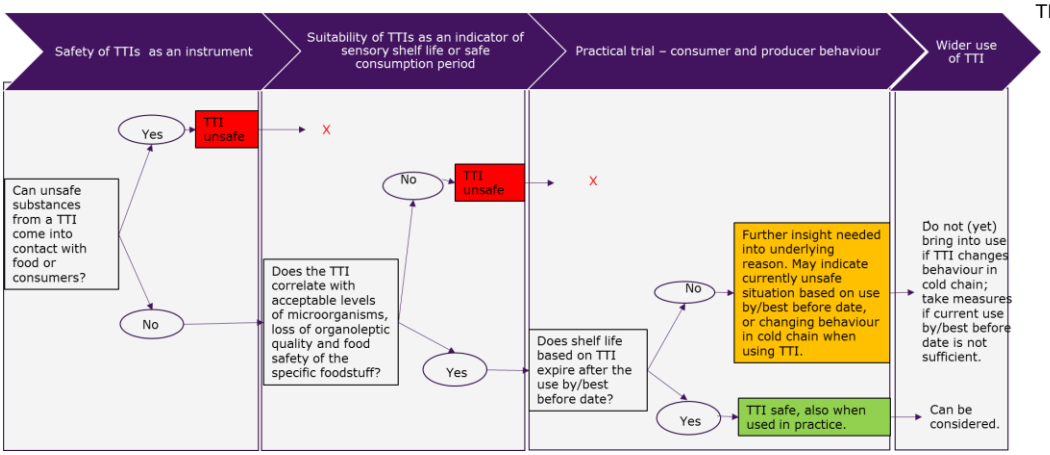

Figure 1: Stepwise approach to assessing food safety associated with a TTI.

These parts can be viewed as stages to be completed in chronological order for each TTI-foodstuff combination, for which the risks are assessed:

1: Which risks are associated with the TTI as an instrument?
2: Which risks are associated with the TTI as an indicator of sensory shelf life or safe consumption period?
3: Which risks are associated with the use of a TTI that has been found safe under consumer and operator conditions in the pilot phase?
4: Which risks are associated with introducing the use of the TTI?

Risk is estimated on the basis of probability (low, moderate, high) and impact (mild, moderate, severe). Each question will focus on TTIs in general. Questions 1 and 2 also assess the risks specific to the Keep-it TTI.

1: Which risks are associated with the TTI as an instrument?

Hazard identification
The active substances in TTIs present a hazard if they are not authorised as starting substances or additives for plastic food contact materials, and the consumer comes into contact with them via the skin during normal use, or if these substances may come into contact with the foodstuff and be ingested during normal use. In addition to contact during normal use, a hazard also arises if the TTI barrier is breached. This can be caused by mechanical failure, if the TTI is exposed to conditions other than those tested, if it is deliberately removed from the food packaging, or in the event of reuse (recycling) of the food packaging material to which the TTI is affixed. If a TTI is included in the recycling process for plastic packaging to be reused for food contact materials, substances that are not on the positive list of Regulation (EU) 10/2011 may end up in plastic food contact materials, albeit in a diluted form.
There are many different types of TTI with different working mechanisms and components, some of which are addressed in this risk assessment. One microbiological TTI evaluated by EFSA contains the components *Canobacterium maltaromaticum* with a plate and acid fuchsin as a colour change indicator (EFSA, 2013). Another TTI is based on growth and metabolic activity of *Lactobacillus*

sakei (Vaikousi et al., 2008) with growth medium (Merck, Darmstadt, Germany) and salts, tested with seven chemical colour indicators. A diffusion-based TTI developed by Kim et al. (Kim et al., 2016) uses isopropyl palmitate.

Specific to Keep-it:
The 'Keep-it' TTI is placed on the outside of food packaging and, for various reasons, can potentially break, meaning that the contents of the indicator can end up on the food or the skin of the consumer. According to the patent, the indicator consists of a chemical reaction between ferricyanide and iron to form a ferricyanide complex:
$3Fe(CN)_6^{4-} + 4Fe^{3+} \rightarrow Fe_4[Fe(CN)_6]_3$
The ferricyanide complex turns blue. A similar blue complex can be formed by adding potassium to the reaction:
$Fe(CN)_6^{4-} + Fe^{3+} + K^+ \rightarrow KFe_4[Fe(CN)_6]_3$
The chemical reaction is time and temperature dependent.
The components are not on the positive list of authorised substances for plastic food contact materials, which means that a functional barrier is required.

Risk: probability
The probability of an unsafe TTI coming onto the market is minimised if the industry submits a dossier in accordance with the guidelines (EFSA, 2009), and if the dossier is found to be suitable during an evaluation by the competent authority of the Member States or by EFSA. This is the case for a number of TTIs (EFSA, 2013).

The TTIs described here are not intended to come into contact with food, but come with a functional barrier that prevents the material from coming into contact with food or consumers. Because of this barrier, the probability of a component of the TTI coming into contact with the food or consumer under foreseeable conditions is low.

The probability of the indicator being damaged during the production process or during use by the consumer and the functional barrier being breached is low. The probability increases if a TTI is not fully tested in extreme situations, such as deliberate removal of the indicator or other situations where the TTI is damaged. During the food production process, the risk of the barrier being breached is minimised on the basis of an HACCP hazard analysis. If packaging bearing a TTI is recycled, there is a high probability that this will lead to contamination during the recycling process and these substances will end up, albeit diluted, in food contact materials.

If the barrier is breached, either unintentionally or deliberately, exposure to the active substances of a TTI occurs. There is a low to moderate probability of ingestion via food or hands or uptake via the skin, depending on the quantities of the active substances present in the TTI and the degree of dilution at which they may end up in recycled material. It is conceivable that children or sellers could deliberately remove a TTI from packaging. This risk can be reduced by stating on the label that the TTI should not be removed and by not using packaging bearing TTIs for recycling into food contact materials.

Risk: impact

Specific to Keep-it:
BuRO does not know whether Keep-it has submitted a dossier in accordance with the EFSA guidelines for authorisation of the TTI. Enquiries with Member States have not yielded this information.
Consumer exposure to iron, potassium, ferricyanide or potassium ferricyanide (reaction product) cannot be determined.
The substances are present at the start of the reaction at a concentration of 0.1 M (=0.1 mol/l) in an unknown volume. The exact concentration of iron, potassium, ferricyanide or potassium ferricyanide in the indicator at the time of any exposure is unclear. This depends on when the indicator on the packaging is broken. To what extent have all components reacted with each other?
The amount of the above substances to which a consumer is exposed or absorbs through the skin if the indicator is broken is then unknown. As the volume of the indicator liquid is unknown, the absolute amount of substance, even at a known concentration, cannot be determined. It is impossible to determine the concentration on the contaminated food and how much of this food the consumer consumes. In the case of dermal exposure, the amount of the substance that ends up on the hands and the amount that is subsequently ingested orally (through hand-to-mouth contact) is also unknown.

EFSA states that, by definition, TTIs with a 'functional barrier' do not migrate in such quantities that the substances have an impact on food safety (contact with the food) or public health (contact with the consumer), or that they can cause unacceptable changes in the food or loss of organoleptic quality.

In the event of deliberate removal, active substances may come into contact with consumers or food and, depending on which active substances are present in the indicator and in what quantities, this can lead to health damage or foodborne illness. In children, the dose at which effects can occur is generally lower than in adults.

The active substances and thus their impact differ from one TTI to another. In general, these are substances and quantities whose impact will be mild, as in the case of the TTI evaluated by EFSA (EFSA, 2013) the components of which are authorised as food additives or colourings, or 'novel food' ingredients. When evaluating a TTI based on acid fuchsin and *Carnobacterium maltaromaticum*, a genotoxic effect of acid fuchsin could not be excluded due to the lack of *in vivo* studies. *Carnobacterium maltaromaticum* was not found to be pathogenic for humans (EFSA, 2013). Vaikousi et al. describe the development of a TTI based on a microbiological mechanism with the bacterium *Lactobacillus sakei,* which is also not pathogenic for humans (Vaikousi et al., 2008).

The impact of active substances of the TTI that can end up in food contact materials through recycling depends on the degree of dilution that occurs during this process.

The impact can be controlled by ensuring that the components of TTIs are substances that have been found to be safe.

Specific to Keep-it (see Appendix 3 for a comprehensive hazard characterisation):

Potassium ferricyanide ($K_4[Fe(CN)_6]$)
Potassium ferricyanide has a low acute oral toxicity. Following dermal exposure in rats, an LD_{50} of 2,000 mg/kg of body weight was reported (ECHA, 2020). When it comes to the use of ferricyanide as a food additive, EFSA has no concerns

regarding genotoxicity. Ferricyanide does not irritate or sensitise the skin (ECHA, 2020).
Iron
Toxic effects can be expected at doses from 20 mg/kg of elemental iron. Ingestion of more than 60 mg/kg is considered a severe case of poisoning (Greupink R. et al., 2015).
Potassium
The toxic dose is highly dependent on kidney function, as with normal kidney function the body is able to eliminate excess potassium quickly. Gastrointestinal side effects are mainly associated with local effects (Nielen J.T.H. & Vanmolkot F., 2016).

Risk characterisation
If the barrier between the TTI and food or consumer is inadequate, active substances can come into contact with the food or consumer. This probability is low during normal use of the TTI and low to moderate in extreme situations such as deliberate removal of the TTI. It is conceivable that children could deliberately remove a TTI from packaging. A pathogenic effect of the active substances in TTIs cannot always be excluded. The probability of exposure leading to illness depends on the amount of the active substance in the indicator, and the impact of this substance. These factors will need to be assessed for each TTI (see also Regulation [EU] 10/2011 and Regulation 450/2009). Generally speaking, the risk associated with TTIs as a product is low because the risk of exposure to active substances is low and the effect mild. In children, a critical limit is reached earlier. One concern is contamination caused by TTI substances in the event that packaging bearing TTIs is recycled into food contact material.

Specific to Keep-it:
It is not possible to estimate the chemical risks in the event of breakage of the indicator due to a lack of data on exposure. This specifically concerns the concentration of the substances present and the absolute quantity in the indicator that could potentially be released in the event of an incident. In addition, information is needed on the consumption amounts of the contaminated foodstuffs and the dermal absorption of the compounds.

Conclusion
- The probability of an unsafe TTI coming onto the market is minimised if the industry submits a dossier in accordance with the guidelines (EFSA, 2009), and if the dossier is found to be suitable during an evaluation by the competent authority of the Member States or by EFSA. This is the case for a number of TTIs (EFSA, 2013).
- Indicators measuring time and temperature can be based on an enzymatic, chemical or microbiological principle.
- In the case of an enclosed TTI that is affixed to packaging, the active substances do not come into contact with the food or the consumer. There is a barrier. These TTIs do not pose a risk under normal circumstances.
- In exceptional cases, this barrier may nevertheless be breached and active substances may come into contact with the foodstuff or the consumer. Where the substances used have been found to be safe, there is no risk. Where potentially unsafe substances are used, food safety risks arise from ingestion through food or hand-to-mouth contact, as well as risks from absorption through the skin. The substances included as active substances vary from one TTI to another. This is not always stated on the TTI.

- Where packaging bearing a TTI is recycled into food contact materials, substances not on the positive list can potentially end up in food contact materials, albeit diluted.
- Keep-it is a TTI with a functional barrier that is not breached under normal circumstances and whose safety can be sufficiently guaranteed if the company includes it in an adequate HACCP. The TTI remains intact at a wide temperature range (-30°C to 90°C). BuRO does not know whether a dossier for Keep-It has been submitted in accordance with the EFSA guidelines.
- In the exceptional case that the barrier of the Keep-it indicator is breached, a risk to food safety and public health cannot be excluded at present because the volume of the active substances is not known. Depending on the volume used, the active substances may pose a risk to consumers.

2: Which risks are associated with the TTI as an indicator of sensory shelf life or safe consumption period?

Hazard identification

Indication of the sensory shelf life or safe consumption period

If a TTI is used in addition to, or as a future replacement for, a best before or use by date, one risk is that the TTI will not correlate with the shelf life (and therefore loss of organoleptic quality or unfitness) or with the safe consumption period (and therefore food safety or harmfulness) of a foodstuff. Loss of organoleptic quality is a hazard for the food itself, namely spoilage, but not so much for the consumer as it is not pathogenic and, moreover, the food is unlikely to be consumed (with some exceptions, see *'aspects relevant to the use of TTIs with a view to food safety'*). Harmfulness of a foodstuff, on the other hand, is a risk to the consumer, and generally not noticeable, which means that the food is likely to be consumed. In terms of the hazard for food safety (and the consumer), as explained above (see *'aspects relevant to the use of TTIs with a view to food safety'*) this mainly concerns microbiologically highly perishable foods where refrigeration or freezing is important to limit or stop the growth of harmful microorganisms to a pathogenic level. A particularly relevant hazard, therefore, is that the TTI may incorrectly reflect the cold chain or freezing chain, or that the cold chain or freezing chain may be interrupted in a way that is not in line with the expected storage conditions as assumed in a shelf life study, and is therefore not tested and the TTI may not measure this situation correctly. Examples include freezing by consumers themselves, or unrefrigerated transport during a heat wave.

Calibration to the properties of the food product

A hazard arises where a TTI is of a general nature and not specifically tailored to the shelf-life related unfitness and harmfulness of the proposed food product. As EFSA also points out in its scientific opinion on date marking, it is important that date marking is carried out on a case-by-case basis (EFSA, 2020). BuRO believes that the same applies to the TTI as a 'flexible date marker'. The growth of microorganisms depends on initial contamination, any bactericidal or bacteria-reducing treatment of the foodstuff, any post-contamination, and then a combination of factors including temperature, pH and a_w value (i.e. water activity in a foodstuff), which should be determined experimentally in order to translate a varying temperature into a corresponding microbial growth curve (Peleg & Corradini, 2011). It is the responsibility of the food business operator to determine the sensory shelf life or safe consumption period. As there are no clear guidelines for this, EFSA has developed a decision tree for date marking of a food (see Appendix 3) (EFSA, 2020), which BuRO also considers relevant to TTIs as a

'flexible date marker'. From a date marking perspective, BuRO agrees with the EFSA approach to TTIs that the key factor is microbial growth during the storage period ('shelf life'), and that bacteria, fungi, yeasts and their toxins (including biogenic amines and histamine) are therefore the relevant hazards (EFSA, 2020). These are relevant hazards, hereafter referred to as 'pathogenic microorganisms', that are present in the foodstuff after processing and packaging and that have the potential to increase during the storage period under reasonably foreseeable conditions.

The case-by-case assessment also applies to changes in recipe, where, for example, less salt or less sugar can affect the sensory shelf life or safe consumption period. Buncic *et al*. mention in their overview on the microbial safety of beef that a microbiological indicator based on *Lactobacillus sakei* can be effectively calibrated to the food product. The assumption here is that growth and metabolic activity is known for the food in question, so that the composition of the TTI can be refined on that basis (Buncic et al., 2014). Yimenu et al. developed a model validated on the basis of microbial growth for monitoring the freshness of packaged chicken meat that has undergone temperature fluctuations (Yimenu et al., 2019).

Indication of relevant spoilage flora

If the TTI does not adequately correspond to the shelf life, a hazard arises in the form of loss of organoleptic quality due to the growth of spoilage organisms. Microbiological spoilage can be caused by the growth or metabolic activity of bacteria, moulds or yeasts. Viruses, parasites and prions do not cause spoilage. Spoilage can be detected by the consumer, for example as visible mould growth or slimy, strong-smelling food. The organisms that cause microbiological spoilage can differ from one foodstuff to another. Spoilage organisms are generally demonstrated by the aerobic plate count. In terms of spoilage, failure by the TTI to correlate with the aerobic plate count criteria is therefore a relevant hazard. It is not a relevant hazard in terms of food safety, since it is not, in principle, pathogenic, also the food is unlikely to be consumed. The relevant spoilage flora for a foodstuff differs from case to case. EFSA has included the following table in its scientific opinion on date marking, which BuRO also considers applicable to TTIs (EFSA, 2020):

Table 1: Major spoilage microorganisms in prepacked foods (Source: (EFSA, 2020)).

Spoilage microorganisms	**Relevant foods/comments**
Aerobic or anaerobic spore-forming bacteria and/or spores, including genera *Bacillus*, *Clostridium*, *Alicyclobacillus*	In heat-treated foods (e.g. mild pasteurisation), spores may be able to survive the heat treatment which may lead to subsequent spoilage during the storage period. In chilled vacuum-packed meat (not heat-treated), 'blown pack' spoilage due to the occurrence and growth of psychrophilic and psychrotrophic *Clostridia* (nonpathogenic).
Lactic acid bacteria, e.g. psychrotrophic genera such as *Leuconostoc, Weissella* and *Lactobacillus*	In vacuum-packed foods and MAP foods (e.g. *Photobacterium phosphoreum* in MAP fish products).
Yeasts e.g. *Candida* spp., *Saccharomyces* spp. and moulds e.g. *Penicillium* spp., *Botrytis* spp., *Alternaria* spp.	Yeasts and moulds mostly dominate the microbiota of a food when the conditions are less favourable for bacterial growth i.e. low pH, a_w (such as fruits, juices, yoghurt, cheese or other fermented foods).

Indication of relevant pathogenic microorganisms (including their toxins and biogenic amines)

When spoilage occurs in food, protein degradation can occur. Biogenic amines, such as histamine, can be formed by certain bacteria (including *Proteus*) from the amino acids released during this process. Fish is highly sensitive to protein degradation and histamine formation (BuRO, 2020a). Although *Proteus* is not in itself pathogenic, and histamine is not a microorganism, histamine is a pathogenic product of a microorganism. Histamine can be present in a foodstuff before spoilage becomes evident. A relevant hazard is therefore that a TTI may not correlate with the criterion for histamine.

If the TTI does not adequately correlate with the safe consumption period, the growth of pathogenic microorganisms constitutes a food safety hazard. This is especially true if the TTI indicates a longer safe consumption period, and for microorganisms that can survive and grow to pathogenic levels during refrigeration:

- At a storage temperature of 4°C*: Bacillus cereus* (minimum temperature 4°C), *Yersinia* (minimum temperature -1.3°C), *Listeria monocytogenes* (minimum temperature -0.4°C);
- At a storage temperature of between 4°C and 7°C*: Staphylococcus aureus* (minimum temperature 7°C), (and histamine formation as mentioned above);
- At a storage temperature above 7°C*: Vibrio* spp. (minimum temperature 10°C), (and histamine formation as mentioned above).

A relevant hazard is therefore that pathogenic levels of the above microorganisms may be reached before the TTI has expired.

In addition to growth at reasonably foreseeable temperatures, these pathogenic microorganisms can grow more easily at higher than recommended storage temperatures. If a TTI does not measure this higher temperature correctly, the hazard increases. On the other hand, if a TTI does measure this higher storage

temperature, it can make an informative contribution to packaged foods and reduce the hazard.

Incidentally, food can already be unsafe at the time of packaging. For some pathogenic microorganisms, the infectious dose is so low that the presence of just one or a small number of cells is already theoretically enough to be pathogenic. Survival or further growth during storage is possible, which means that these pathogenic microorganisms remain a relevant hazard including in relation to a TTI. This applies to *Clostridium botulinum* (minimum temperature 3.3°C), *Salmonella* (minimum temperature 5.2°C), STEC (minimum temperature 6.5°C), and *Shigella spp*. (minimum temperature ≥6.1°C) and their toxins (Schmid-Hempel & Frank, 2007; BuRO, 2016).

It should be noted that legal food safety criteria do not exist for all the relevant hazards mentioned above. Such criteria do exist for *Bacillus cereus, Listeria monocytogenes, Salmonella,* STEC*, Staphylococcus aureus* and histamine, but not for *Yersinia* spp, *Vibrio* spp, *C. botulinum* and *Shigella*.

EFSA provides the following non-exhaustive table in its overview of relevant pathogenic microorganisms for date marking per product group with regard to foodstuffs for which cooling relative to ambient temperature is required:

Table 2: Non-exhaustive summary of pathogenic microorganisms of relevance for date marking for different perishable food categories (including raw and processed prepacked foods) (Source: (EFSA, 2020)).

	Group Genera/species	Food category of concern	Examples of food type
Gram-negative (enteric) bacteria	**Mesophilic**		
	Salmonella spp., pathogenic *E. coli*	Meat and products thereof	Raw pork meat, raw beef
		Fish and seafood	Shellfish
		Fruits and vegetables	Fresh cut/RTE vegetables (sprouts, spinach, …) and fruits
		Milk and dairy products	Fresh/cottage cheese, raw milk
		Prepared/mix food	Prepared salads, sandwiches
	Psychrotrophic		
	Yersinia enterocolitica	Meat and products thereof	Raw minced meat
Gram-positive bacteria	**Non-toxicogenic** *Listeria monocytogenes*	Prepacked raw RTE food	Salads, fruit juices, fresh cut vegetables and fruits
		RTE food exposed to contamination after a processing step causing microbial inactivation	Cooked meat products, smoked fish, soft/semi-soft and fresh/ cottage cheese
	Toxicogenic **Non spore forming** *Staphylococcus aureus*	Meat and products thereof	Cooked meat products
		Fish and seafood	Cooked fish products
		Cheese and dairy products	Raw milk cheese, soft cheese
		Bakery products	Cream-filled pastries, pies
		Prepared meals	Fish dishes, meat dishes, cheese containing dishes
	Spore forming aerobic *Bacillus cereus* (Diarrheic and emetic)	Food of non-animal origin, particularly heat treated	Cooked dishes/meals containing pasta or rice, such as tabbouleh, rice salad, semolina, rice pudding
		RTE prepared/mix food/meals (REPFED)	Cooked vegetables and potatoes, vegetable puree Meat-based meals with non-animal components (sauce, vegetables)
		Milk and dairy products	Pasteurised milk and dairy products and desserts
	Spore-forming anaerobic psychrotrophic non-proteolytic *Clostridium botulinum* **mesophilic** proteolytic *Clostridium botulinum*	Reduced atmosphere packed food, particularly heat treated (REPFED)	Salted fish, cooked meat products (pâté, sausages), hummus
		Seafood and meat products	Canned fish (sardines, anchovies, tuna) and meat products (corned beef, pâté)

Note: Foods exempt from the requirements to indicate a 'best before' date or covered by other EU provisions imposing other type of date marking, and excluded from this opinion, are listed in Appendix A.
RTE: ready-to-eat; REPFED: refrigerated (minimally) processed foods of extended durability.

In summary, BuRO has drawn up the following overview of relevant pathogenic microorganisms according to the different approaches, whereby EFSA (EFSA, 2020) states that the list is non-exhaustive in relation to use by/best before dates, and BuRO has made some additions to the list in relation to TTIs:

Table 3: Relevant pathogenic microorganisms in relation to date marking of perishable foods.

Hazard	Relevant according to BuRO for TTI date marking	Relevant according to EFSA for date marking*	Legal criteria
Growth required during safe consumption period			
Histamine	Yes	No	Yes
Bacillus cereus	Yes	Yes	Yes
Yersinia enterocolitica	Yes	Yes	No
Listeria monocytogenes	Yes	Yes	Yes
Staphylococcus aureus	Yes	Yes	Yes
Vibrio spp.	Yes	No**	No
No growth required during safe consumption period			
Clostridium botulinum	Yes	Yes	No
Salmonella	Yes	Yes	Yes
STEC	Yes	Yes	Yes
Shigella spp.	Yes	No***	No

*list is not exhaustive
**EFSA did not consider *Vibrio parahaemolytisus* relevant to best before/use by dates due to its growth in food matrices between 12°C and 15°C. BuRO points out that the TTI can have added value precisely at higher than recommended storage temperatures.
***EFSA states that *Shigella* spp. is controlled if other specified pathogenic microorganisms are also controlled.

Specific to Keep-it:
Keep-it works with a temperature range from -30°C to 90°C. At a constant temperature of 4°C, the colour development progresses to 50% after 19 days, and to 93% of the indicator after 76 days, which means that it can be used for refrigerated foodstuffs, but possibly also for non-refrigerated foodstuffs. The correlation of the indicator with the shelf life of the proposed food products (chicken drumsticks, tilapia fillets) was tested on the basis of aerobic plate count, *Enterobacteriaceae* and *Pseudomonas* spp.
Recipe changes are included in the HACCP to be passed on by the producer to Keep-it. Such changes should also be included in the HACCP for the proposed food product by the food producers.

Risk: probability

Indication of the sensory shelf life or safe consumption period

The probability of the TTI giving an incorrect indication of the shelf life (best before date) or safe consumption period (use by date) is high if the TTI for the packaged food has not been sufficiently tested in relation to the recommended storage temperature, or if the food is stored at a different temperature not evaluated in the shelf life study. Although this applies mainly to refrigerated or frozen food, it also applies, to a lesser extent, to food that can be stored unrefrigerated (with a best before date). However, temperature is not the only factor to determine the shelf life of foodstuffs that are to be stored unrefrigerated.

The probability of a TTI giving an incorrect indication of sensory shelf life or safe consumption period also increases if the TTI has not been sufficiently tested under extreme conditions, such as insufficiently refrigerated transport during a heat wave, or freezing by the consumer. TTIs based on a solid-state polymerisation reaction are known to be stored deep frozen to limit progression starting from production (Koutsoumanis & Gougouli, 2015), which indicates that this reaction is minimised during freezing. TTIs based on a microbiological principle are stored frozen to prevent microbial growth and are activated by thawing (Koutsoumanis &

Gougouli, 2015). These TTIs therefore reflect microbial growth but not any chemical or physical spoilage that may occur in the frozen state. In the diffusion-based TTI, the speed of diffusion can be selected within a temperature range of -15°C to 26°C (Realini & Marcos, 2014). On the one hand, this TTI lends itself to use for frozen food, but on the other hand, 26°C does not reflect the temperature that can be expected during unrefrigerated transport in a heat wave. The temperature to which foodstuffs may be exposed during a heat wave can reach 40°C in a car, and this temperature falls outside the test ranges described in the literature.

In addition to the above extremes, the probability of a TTI incorrectly indicating a longer shelf life increases if realistically expected refrigerator temperatures are not tested. Such temperatures may be higher than currently assumed in shelf life studies. For example, a Dutch study shows that the refrigerator temperature is regularly higher than this recommended temperature, namely between 3.8 and 11.6°C. In two-thirds of cases (21 out of 31), the refrigerator temperature was even higher than the maximum temperature of 7°C recommended by the Dutch Consumers' Association. Although this was a small study, these temperatures are consistent with those in a larger study in eight European countries, where temperatures ranged from 5.3 °C to 10.4 °C, and maximum temperatures from 9.3°C to 21.8 °C (EFSA, 2020).

Calibration to the properties of the food product

The probability of a TTI being incorrectly calibrated to the food product is high if the food business operator has not included the TTI in the date marking process for the food in question. The probability of a TTI of a general nature, such as one with a preset default expiry time of 10 days at 4°C, being insufficiently calibrated to a specific foodstuff is moderate. The probability of a TTI being insufficiently calibrated to the foodstuff increases in the event of recipe changes that affect its shelf life. The probability of recipe changes is high, given the government's initiatives (https://www.rijksoverheid.nl/onderwerpen/voeding/gezonde-voeding/minder-zout-verzadigd-vet-en-suiker-in-voeding).
It is reasonable to expect consumers to freeze food. The shelf life of a food after freezing and defrosting also depends on the composition of the food. Freezing by the consumer may result in an unsafe food product if the TTI does not indicate that the shelf life has expired.

Incorrect indication of relevant spoilage flora

The probability of a TTI not corresponding to the microbiological criterion for spoilage flora is high if the food business operator has not included the TTI in the date marking process for the food in question. The probability of an incorrect TTI indication of spoilage flora leading to illness is low, because the loss of organoleptic quality will cause the consumer to decide not to consume all or part of the food, and moreover, spoilage flora are not pathogenic (with a few exceptions).

Indication of relevant pathogenic microorganisms

The probability of the above-mentioned pathogenic microorganisms reaching pathogenic levels before a TTI has expired depends on the storage temperature and the calibration of the TTI to that temperature in relation to the dynamics of the microorganisms in question:

- at a maximum recommended refrigeration temperature of 7°C *Yersinia* spp., *S. aureus, B. cereus*, *L. monocytogenes* and histamine formation*;*
- at higher temperatures where faster growth can be expected: *B. cereus*, *S. aureus, Vibrio* spp. and histamine formation.

Although additional growth is less relevant for pathogenic microorganisms that are already pathogenic without growth, they can grow to larger numbers during the sensory shelf life or safe consumption period, which increases the risk of illness or more severe illness following ingestion. This applies at the recommended refrigerator temperature for *Salmonella,* STEC/EHEC, *C. botulinum*, and at higher temperatures for *Shigella*. The longer the food is stored prior to consumption, and the higher the storage temperature above the recommended storage temperature, the greater this probability becomes. If a TTI extends the shelf life, both correctly and incorrectly if the TTI is insufficiently calibrated to the dynamics of microorganisms, this probability therefore increases.

The literature shows that the correlation of a TTI with microbial growth is not always found, or only for certain temperature ranges. The review by Sohail *et al*. (Sohail et al., 2018) states that a diffusion-based TTI could only be used correctly as an indicator of microbial growth, namely total aerobic plate count, in pasteurised angelica root juice at a temperature of 13°C or higher (Kim et al., 2016). The minimum growth temperature of the most relevant pathogenic microorganisms is lower, and no correlation with microbial growth was observed.

Although the legislation distinguishes between foods on the basis of whether or not they need to be heated prior to consumption, EFSA (EFSA, 2020) states that this is in fact irrelevant when choosing a best before or use by date, as cross-contamination can occur. A consumer may also consume the food insufficiently heated, contrary to the instructions. BuRO shares this view, also in relation to introducing the use of a TTI. The probability of exposure to spoilage organisms and pathogenic microorganisms is increased by the consumption of food that is not properly cooked or raw. The probability of fresh chicken meat being insufficiently heated before consumption is low, but conceivable in situations such as barbecues. The probability of consuming fresh fish raw is moderate, as in the case of sushi, for example. In addition, the Fish Chain Risk Assessment found that consumers are served prawns uncooked in restaurants (Hoel et al., 2015; Foodlog, 2019). Non-compliance with the instruction to 'heat prior to consumption' increases the risk of exposure to unsafe food.

Pathogenic microorganisms can only grow to pathogenic levels if the pathogenic microorganisms are already present in the foodstuff at the time of packaging. The probability of growth to pathogenic levels increases the higher the initial contamination, and decreases due to the fact that food products are required to meet standards immediately after production (Regulation [EC] No. 2073/2005 on microbiological criteria for foodstuffs). This is described in NVWA Info Sheet 85 as an interpretation of the aforementioned Regulation, in relation to the obligations of food producers including HACCP. EFSA has also set out a science-based interpretation of the Regulation in terms of aspects relevant to date marking (EFSA, 2020).

Specific to Keep-it:
The version of the TTI used has a default expiry period of 10 days at 4°C. Different temperature profiles have been tested for two food products, namely chicken drumsticks and tilapia fillet, which also included a temperature shock. Tests show that in 6 out of 16 tested temperature profiles, Keep-it incorrectly indicated a longer storage period based on aerobic plate count, *Enterobacteriaceae* and *Pseudomonas* spp.
It is stated that *Salmonella* has not been detected in monitoring programmes in raw materials or packaged fish in recent years, and that monitoring of high-risk raw materials and final product is carried out for the proposed food products (Bouwman et al., 2019). The trial is based on food products that need to be heated prior to consumption, which reduces the chance of survival of the most

relevant pathogens such as *Listeria* spp. However, cross-contamination may occur or the food may be consumed insufficiently heated, meaning that other pathogenic microorganisms may still be present. Moreover, histamine is heat-resistant. The correlation of Keep-it with histamine formation was not tested. With regard to potential histamine formation, it is stated that tuna from the freezer is first checked for histamine to ensure that the level is sufficiently low during production (Bouwman et al., 2019).

Risk: impact
The impact of an incorrect shorter sensory shelf life or safe consumption period based on a TTI is the discarding of safe and unspoilt food. Although this does not have an adverse effect on food safety, it does encourage food waste.

The impact of an incorrect longer sensory shelf life or safe consumption period based on a TTI is the possibility of spoiled or harmful food. If spoilage has not yet occurred, the impact is that a harmful foodstuff may be consumed and cause illness. For pathogenic microorganisms that need to grow to be pathogenic, the impact is that a previously safe food becomes unsafe. The following hazards are relevant here: *L. monocytogenes (*-0,4°C, 10^3-10^4 cells), *Yersinia* spp. (-1.3°C, ≥10^4 to 10^8 cells), *B. cereus (*4°C but 10°C for toxin formation, 10^5 cfu/g, spore-forming), histamine formation (particularly from 4°C), *S. aureus (*7°C but 10°C and >10^5 cfu/g for toxin formation), *Vibrio* spp. (10°C, ≥10^3 cells to 10^6 cells). In the case of foodstuffs that were already unsafe at the time of packaging, the impact is that the dose is higher and the illness can therefore be more serious. This applies to *Salmonella*, STEC, *C. botulinum* toxin and *Shigella*. See Appendix 4 for a comprehensive hazard characterisation. The hazards that are actually relevant depend on the foodstuff in question, as is also the case with best before/use by dates (EFSA, 2020).

Specific to Keep-it:
During the tests on both proposed food products, the aerobic plate count and *Enterobacteriaceae* criterion was exceeded in a number of situations. The current version of the TTI is therefore inadequately calibrated to the foodstuffs in question and may have a negative impact on food safety. There is an anticipated impact on all pathogens relevant to the food product.

Risk characterisation
The food safety risk associated with a TTI that is insufficiently calibrated to the proposed foodstuff is assessed as moderate based on probability (moderate) and impact (mild for the general population, moderate for vulnerable populations). The food safety risk associated with a TTI that, like best before and use by dates, is sufficiently calibrated to the proposed foodstuff is estimated as low based on probability (low) and impact (low for the general population, moderate for vulnerable populations). The risk is estimated to be moderate (general population) to high (vulnerable populations) where consumers do not follow the instructions on the label, or do not pay sufficient attention to cross-contamination.

Control measures
Food business operators are responsible for determining the sensory shelf life or safe consumption period to ensure that a food product remains safe during the indicated storage period. In the case of the TTI, this responsibility lies with the company that affixes the TTI to the packaging. In the case of use by and best before dates, this is a legal obligation. In order to control the risk associated with any extended shelf life, such legal responsibility also needs to be established for the TTI.

The criteria and the study referred to in Regulation (EU) 2073/2005, of which the NVWA's interpretation is described in Info Sheet 85, and which are currently used for determining safety and shelf life on the basis of use by and best before dates, will also control the risks associated with TTIs. EFSA has developed a science-based decision tree for this purpose. The risk associated with TTIs is mitigated by including the TTI as a 'flexible date marker' in this decision tree, in addition to the use by or best before date (Appendix 3). For foods in which microbiological growth or toxin formation is possible, it is important from a food safety perspective that the TTI is included in the assessment as one of the foreseen conditions, namely a potentially extended safe consumption period indicated by the TTI that corresponds to the dynamics of the relevant pathogenic microorganisms for this food.

The decision tree distinguishes between four different categories of food, whereby BuRO has identified potential for the assessment of the TTI, namely:

I. frozen food (best before date)
In the case of these foodstuffs, the extent to which the TTI is able to monitor the maintenance of the freezing chain, and thus the growth of microorganisms, can be assessed for the TTI. For TTI mechanisms that continue during freezing, it can be investigated whether the TTI is also suitable for indicating chemical or physical spoilage.

II. food that can be stored at ambient temperatures (best before date)
In the case of these foodstuffs, a TTI can be assessed in relation to extreme temperatures, bearing in mind that temperature is only one of the factors involved in determining the shelf life of these foods.

III. food in which foreseeable storage conditions do not allow the growth of pathogens or the production of toxins (best before date)
In the case of these foodstuffs, a TTI can be assessed as an indicator of relevant spoilage flora, noting that these may be different spoilage flora than those to which legal criteria apply.

IV. food in which pathogen growth or toxin production can be expected under foreseeable storage conditions (use by date).
In the case of these foodstuffs, a TTI must be assessed for relevant pathogens, noting that this may include more relevant pathogens than those to which legal criteria apply.

Specific to Keep-it:
Keep-it operates across a wide temperature range. The correlation with food safety was tested with a default calibration of 4°C with a 10-day expiry period. The correlation with food safety for the proposed foods has not been sufficiently demonstrated, and in some cases results in an unsafe food product. The correlation with histamine formation has not been tested, although histamine formation is expected to occur. This poses a risk to people who are sensitive to histamine. In terms of foodstuffs, fresh fish, smoked fish and meat products pose the greatest risk, with the risk of *Listeria* spp. applying mainly to ready-to-eat food and to vulnerable populations, and the risk of histamine applying mainly to fish and to people with histamine sensitivity.

Control measures: Keep-it will not be used for ready-to-eat food at this time. If this is the case, the producer indicates that NVWA Info Sheet 85 will be followed (Bouwman et al., 2019). The study design states that the supermarket chain will affix the TTI to the proposed food products, making the supermarket chain responsible for safety during the shelf life indicated by the TTI.

Conclusion

- Microorganism growth limits the sensory shelf life or safe consumption period of perishable foods. Where this involves the growth of spoilage microorganisms, it leads to unfitness that is noticeable to the consumer, meaning that all of part of the foodstuff is not consumed. Where it involves the growth of pathogenic microorganisms, this leads to harmfulness that is usually not noticed by the consumer. It is therefore likely that a harmful but unspoilt foodstuff will be consumed and lead to illness.
- The relevant pathogenic microorganisms differ for each foodstuff (fish, chicken, meat, dairy, ready meals, etc.). Microbiological criteria have been drawn up for this purpose (in Regulation [EU] 2073/2005 Article 3; the Processing of Food [Commodities Act] Decree [WBBL]; Info Sheet 85), however, more pathogenic microorganisms may be relevant for a foodstuff than those to which legal criteria apply. To this end, EFSA has developed a useful, science-based decision tree for date marking (EFSA, 2020). BuRO views the TTI as a flexible date marker that can also be assigned to a foodstuff using this decision tree.
- Refrigeration is a measure used to restrict microbial growth, and freezing to stop it. Because temperature is a critical factor in relation to the sensory shelf life or safe consumption period (time), TTIs can provide meaningful information on the safety of a foodstuff provided that the TTI endpoint correlates with the dynamics of microorganisms in the proposed food product at the foreseeable time-temperature conditions for the foodstuff (refrigeration, shopping, transport and freezing).
- The probability of the TTI correlating poorly with food safety is moderate if the TTI has not been sufficiently tested under reasonably foreseeable conditions. Consumers store food at a higher temperature than the recommended 4°C or 7°C. This allows the growth of spoilage organisms and pathogenic microorganisms during the storage period. There is also a high probability of recipe changes, such as less salt or sugar, to which the sensory shelf life or safe consumption period based on a TTI will need to be adjusted. The same applies to shelf life based on a best before date and safe consumption period based on a use by date.
- Currently, this correlation of TTI endpoints with microbial growth for relevant temperature ranges is not always observed. This means that, at present, some TTIs incorrectly suggest that a product is safe and would lead to an unsafe situation if the TTI exceeds the use by date and, when put into practice, if the product were to be consumed based on the indication given by the TTI.
- It is possible to see, feel and smell loss of organoleptic quality, which means that the food is generally not consumed and therefore does not cause illness. This is applicable by legal definition to foodstuffs that bear a best before date. The food safety risk associated with a TTI is mainly caused by foods bearing a use by date where the TTI extends the indicated safe consumption period, while this TTI does not sufficiently reflect the dynamics of the microorganisms. This is especially true of pathogenic microorganisms that need to grow in order to be pathogenic and that can grow at a realistically expected refrigerator temperature. Based on Terpstra *et al*., the realistically expected refrigerator temperature is between 3.8 °C and 11.6 °C. The main relevant pathogenic microorganisms (including toxins and biogenic amines) are *L. monocytogenes* and *Yersinia* spp., which can grow at the recommended refrigerator temperature of 4 °C; to some extent histamine*(Proteus*), which can be formed at the recommended maximum refrigerator temperature of 7°C; and to a lesser extent *B. cereus, S. aureus* and *Vibrio* spp., which grow or produce toxins at temperatures higher than the

recommended maximum temperature of 7°C. The estimated severity of the risk is low for the general population and moderate for vulnerable populations.
- The presence of *Salmonella* and STEC is already a food safety risk, irrespective of whether or not the TTI is accurate. A longer sensory shelf life or safe consumption period indicated by the TTI can lead to growth at the expected refrigerator temperature over a longer period of time, potentially resulting in more serious infections. This risk increases if the consumer consumes the food at the end of the indicated shelf life.
- The food safety risk posed by a TTI that is insufficiently calibrated to the proposed food product is estimated as moderate based on probability (moderate) and impact (mild for the general population, moderate for vulnerable populations). The food safety risk associated with a TTI that, like best before and use by dates, is sufficiently calibrated to the proposed foodstuff is estimated as low based on probability (low) and impact (low for the general population, moderate for vulnerable populations).

3: Pilot use of TTIs alongside a static expiry date

The pilot phase will look at the additional risk when the consumer is faced with a TTI on a food product that also bears a use by or best before date. The assumption is that the TTI as an instrument has been found to be safe and that it is substantiated that the TTI is a good indicator of the sensory shelf life or safe consumption period under foreseeable storage conditions, as also needs to be substantiated by the food business operator in the case of use by or best before dates.

Hazard identification

TTIs are in conflict with the law

Where the TTI indicates a shelf life greater than or equal to the use by/best before date, this does not present any relevant food safety hazards (assuming the indication is correct and the instrument is safe). However, it does conflict with the legally enshrined indication of unsafe food products, namely the use by date, which requires the label to state that the food must not be consumed after this date[7]. However, if this is made legally possible, food can be safely consumed for a longer period of time in the case of a TTI that expires after the use by date. This can help to prevent food waste if the consumer wishes to consume the food product towards the end of its actual shelf life.

TTIs are added to food products at a later stage

The regulator holds the food business operator that affixes the TTI to the food product responsible for the safety of the food during the sensory shelf life or safe consumption period indicated by the TTI *(personal information provided by Coen vd Weijden*). A hazard here is that this responsibility is unclear and the operator of any subsequent link may not carry out the necessary studies.

TTIs bring currently unsafe situations to light

Where the TTI indicates a shorter sensory shelf life or safe consumption period, this shows that the current use by/best before date does not correlate with food

[7] Section 15, paragraph 3, of the Preparation and Handling of Food (Commodities Act) Decree (*Warenwetbesluit Bereiding en behandeling van levensmiddelen*):
3. Without prejudice to the applicable requirements regarding the labelling of food products, the packaging of the food or beverages referred to in paragraph 2(a) shall bear storage conditions stating, among other things, that the product must be consumed within a certain number of days after purchase, but never later than the pre-specified date.

safety. Reasons for this can include extreme handling during the cold chain, by the operator, during transport or by the consumer. Where a TTI indicates a shorter safe consumption period than the use by date, it is important from a food safety perspective to gain further insight. If this scenario occurs at normal temperature profiles, the current situation for that particular food product is unsafe.

TTIs lead to consumer confusion

During the pilot phase, one hazard is that consumers may not understand the TTI or may struggle with two different expiry dates on a product. Although it is debatable whether a TTI legally falls under the definition of 'voluntary food information' (see '*Use of TTIs on food products*'), the hazard is that these coexisting sources of information are confusing for consumers.

TTIs provoke abnormal behaviour

Another hazard arises where food business operators or consumers do not follow instructions on the packaging, such as storage advice or heating prior to consumption. This hazard can also be triggered by consumers or food businesses relying on the performance of the TTI, resulting in untested temperature profiles. For example, the food may be kept outside the refrigerator for a longer period, because this is cheaper for the operator, and the TTI takes this into account in terms of shelf life. Or, after freezing and defrosting, the food is kept in the refrigerator for a while because the TTI indicates that the shelf life has not yet expired. Although this hazard also applies to use by/best before dates, if a TTI indicates a longer shelf life, this hazard may become more relevant to pathogenic microorganisms that can grow and be present at higher levels in the foodstuff during the extended period at the storage temperature.

Changing behaviour by combating food waste

Another hazard arises where combating food waste leads to changes in the behaviour of operators or consumers. A consumer who is conscious of food waste may be more likely to purchase products just before or at the end of the shelf life than other consumers, for instance because these foods are discounted, or offered through an app with the aim of reducing food waste. One example is the 'Too Good To Go' app (https://toogoodtogo.nl/nl). As a result, the 'temperature shock' caused by shopping occurs at the end rather than the beginning of the shelf life. A consumer then also has a larger quantity of foodstuffs close to the expiry date. One hazard here is that a number of foodstuffs may be consumed after the expiry date.
A related hazard is where food is sold or given away via alternative routes after the best before or use by date.

Risk: probability

TTIs are in conflict with the law

The probability of TTIs being in conflict with the law is high. This probability is reduced by granting an exemption from Section 15, paragraph 3, of the Preparation and Handling of Food (Commodities Act) Decree.

TTIs are added to food products at a later stage

The probability of unsafe food is low if the TTI is affixed to the packaged food at the beginning of the production phase, and moderate if the TTI is applied by a food business operator at a later stage. The probability of the TTI indicating a shelf life of safe consumption period that is too long is further increased where

changes are made to recipes earlier in the chain and such changes are not communicated to later stages of the chain. The probability is reduced if the necessary date marking studies have been carried out, with TTI being assessed as a flexible date.

TTIs bring currently unsafe situations to light

Where the TTI correctly indicates a shorter shelf life than the use by/best before date, the risk of illness is reduced if the consumer relies on the TTI.

TTIs lead to consumer confusion

The results of a trial by a meal box delivery service, supplied by the Ministry of Health, Welfare and Sport, indicate that, although based on self-reporting, consumers understand the Keep-It TTI and do not find it confusing.

TTIs provoke abnormal behaviour

There is insufficient information on the probability of the TTI provoking abnormal behaviour, such as storing the product outside the refrigerator for longer than recommended. A pilot study can provide insight in this area. Where consumers have confidence in the performance of the TTI, there is a risk that temperature profiles not tested in the feasibility studies may occur. The dynamics of microorganisms can then be different than expected. At present, not enough is known about the extent to which any changing behaviour contributes towards the probability of unsafe food. Further insight is needed into the extent to which this can increase the risk of disease.

Changing behaviour by combating food waste

With regard to food waste, Manning et al. state in their review that consumers consider throwing away food to be undesirable behaviour (Liegeard & Manning, 2020). It is not currently possible to determine from the literature how a food waste-conscious consumer approaches instructions such as storage advice and best before or use by dates. Where consumers regularly take advantage of offers or use apps to combat food waste, the risk of exposure to pathogens increases. In the case of perishable foodstuffs, failure to observe the instructions and date increases the risk of illness.
The probability of foodstuffs being sold after the best before or use by date in the Netherlands is low. EFSA states that some European countries, namely Greece, Norway, and Sweden, allow sales after the best before date. In Norway, food with a 'short best before date' can be sold through special internet shops for a lower price. In Sweden, these products can be sold, potentially frozen, under the responsibility of the seller. In Greece, such products are assigned a 'past best before date', with Greek law stipulating how long a foodstuff can be consumed after the best before date (one week for a day-month date; one month for a month-year date; three months for a year date). EFSA states that Greece makes no reference to any scientific evidence to support this approach (EFSA, 2020).

Risk: impact

TTIs are in conflict with the law

The impact of an accurate TTI that has been found to be safe exceeding the use by date, which means that the food product must not be consumed according to the law, has no effect on food safety. However, it may have the effect of encouraging food waste if consumers follow the instructions on the label and throw away safe food.

TTIs are added to food products at a later stage

If a TTI is affixed at a later stage in the chain, and the necessary studies have not been carried out, the TTI may incorrectly indicate a longer shelf life, which could lead to illness as a result of the consumption of harmful foods. This impact is estimated to be mild for the general population and moderate for vulnerable groups.

TTIs bring currently unsafe situations to light

If the shorter shelf life based on the TTI is due to an atypical situation in the cold chain, the impact is that the TTI leads to less illness. If the cold chain did not show any deviations, the impact is that, at the present time, the use by/best before date is unsafe and possibly responsible for part of the currently known burden of disease.

TTIs lead to consumer confusion

No impact is to be expected from misunderstanding a correctly functioning TTI that has been found to be safe.

TTIs provoke abnormal behaviour

The impact of not following the instructions on the packaging is that harmful foodstuffs may be consumed. The estimated severity of the impact is mild for the general population and moderate for vulnerable groups.

Changing behaviour by combating food waste

Based on the literature, there is currently insufficient information available on the impact of a temperature shock in the final stage on the shelf life on food safety of the food product, or of frequent exposure to foods that are at the limit of their sensory shelf life or safe consumption period.

Risk characterisation

Where a TTI is suitable to be admitted to the pilot phase, it can improve food safety. A risk may arise where (i) the TTI is affixed to the food product at a later stage without any studies having been carried out at this later stage, or where recipe changes are not communicated to this later stage, (ii) the TTI provokes abnormal consumer or operator behaviour due to confidence in its performance and failure to follow the storage recommendations on the packaging, and (iii) food waste-conscious consumers are frequently exposed to foods that are at the limit of their sensory shelf life or safe consumption period. While i) and iii) also apply to best before/use by dates, ii) is specific to the TTI due to an increased probability of unexpected, and therefore untested, temperature profiles. The correlation of the TTI with food safety may be insufficient outside these tested temperature profiles, which can result in unsafe food. If a TTI is brought into use that does not show sufficient correlation with food safety under extreme conditions, the additional risk will be limited to people consuming the food after the use by date.

Control measures

In order to reduce the risk associated with TTIs affixed at a later stage, it is necessary to conduct studies at this later stage to ensure safety during the shelf life indicated by the TTI. The EFSA science-based decision tree can be used for this purpose (Appendix 3a). A critical point here is that the food business operator communicates recipe changes to the operator later in the chain. The risk of consumers not following instructions can be reduced by clear instructions during the pilot. However, it is a risk that currently also applies to foodstuffs bearing a best before or use by date, which can be further controlled through education.

Evaluation of pilot phase
In order to expand the use of a TTI after the pilot phase, it is important to evaluate the pilot phase. This involves:

- gaining insight into consumer and operator behaviour when using a TTI, and whether this poses a risk to food safety;
- gaining insight into the extent to which instructions are understood and followed by average consumers, food waste-conscious consumers and vulnerable populations;
- further research if a TTI indicates a shorter sensory shelf life or safe consumption period, i.e. brings any currently unsafe situations to light.

If the evaluation of the pilot phase reveals that instructions are clear, followed, and operator and consumer behaviour when using the TTI does not lead to consumption of unsafe food, the TTI can be used more widely.

Conclusion

- The use of an appropriately validated TTI as a safety indicator with a positive outcome (i.e. a safe food during the indicated storage period) does not pose a risk to food safety and can help to reduce food waste. Moreover, a TTI can even help to improve food safety if the use of the TTI shows that the safe period for consumption is shorter than the use by date.
- The use of both a TTI and a use by or best before date can potentially be confusing for consumers if there are two different expiry dates. However, initial studies do not indicate that this is the case, although it should be noted that these studies were based on self-reporting.
- The food business operator that affixes the TTIs to food products is responsible for the safety of the food until the best before or use by date, and must be able to demonstrate its safety based on studies.
- Once the use of a TTI is permitted as part of a practical trial, operator and consumer behaviour will start to play a role in the emergence of food safety risks. For example, purchase by the consumer on one of the last days before expiry of the use by date, as a discounted item or as part of a 'Too good to go' package whereby a package of food products is purchased on the label date. Or consumers and operators rely on the TTI to such an extent that the food is kept out of cold storage for some time.

4: Introducing the use of TTIs and/or extension of their use to other food products, possibly without a static expiry date

Hazard identification
If a TTI were to be introduced for wider use, and/or potentially as a replacement for the use by/best before date, a number of additional relevant hazards have been identified on the basis of the current risk assessment:

- TTIs not enshrined in law in terms of the responsibility of the food operator during the shelf life;
- social trends affecting shelf life and consumer behaviour for which the TTI has not been tested (also applies to use by/best before dates);
- changes in the composition of foodstuffs that affect their shelf life and that do not result in recalibration of the TTI (also applies to use by/best before dates).

If the evaluation of the pilot phase shows that the TTI indicates a longer shelf life than the use by/best before date, and the use by/best before date would be abandoned, the additional hazard arises that the TTI will provoke a change in operator behaviour. Currently, refrigeration to prevent growth is an important control step, and hygiene codes define fixed, relatively short shelf lives on this basis. In the case of a TTI, there is a risk that the operator will opt for a shorter shelf life by reducing or eliminating cooling and thus saving on cooling capacity.

Risk: probability
There is a high probability that the fact that TTIs are not enshrined in law will lead to harmful food products. This is especially true for perishable foodstuffs, but also for foodstuffs that can be kept chilled for longer. The probability is reduced if an operator, despite the absence of a legal obligation, takes responsibility for placing a safe food product on the market in terms of safe consumption period and shelf life.

There is a reasonable probability that the composition of a food will change in a way that shortens its shelf life. Trends towards less salt, less sugar, less processed increase this probability, which can lead to an unsafe food if the TTI is not adjusted accordingly. Trends such as 'raw cooking' and 'slow cooking', where it is likely that a food will be consumed undercooked (for the purpose of microorganisms), also pose a risk. This probability also applies to use by/best before dates. It is also conceivable that future trends could affect food safety, for example through use other than that for which a food or product is intended, i.e. 'improper use' such as the consumption of flowers.

If consumers do not receive proper information about correct use and interpretation of the shelf life according to TTIs, there is a reasonable probability of harmful foodstuffs being consumed. Examples include following instructions for storage and use with regard to refrigerated storage, freezing, thawing, or purchasing a package of perishable foodstuffs that is on the last day of its shelf life ('Too Good To Go'), but may be too much to consume on the same day.

Operators are highly likely to change their behaviour if it saves costs and provides a similar shelf life as is currently the case based on use by/best before dates.

Risk: impact
The impact of unsafe food as a result of the fact that TTIs are not enshrined in law, incorrect use or misinterpretation, or inadequate adjustment of the TTI in response to changes in the composition of the food, is illness due to consumption of harmful food. The impact is estimated to be mild for the general population and moderate for vulnerable groups.

Changing operator behaviour does not have a negative impact on food safety if the TTI continues to correlate with safety of the food product, however in this case the TTI does not help to prevent food waste. Where the changed behaviour results in temperature profiles not included in the shelf life study, and at which microorganisms show different dynamics, the changed behaviour may result in a harmful food.

Risk characterisation
If TTIs are not enshrined in law, there is a foreseeable risk of harmful food in the case of perishable foodstuffs that are not or insufficiently heated prior to consumption, with the impact being more severe in vulnerable populations *(Listeria* spp., *Yersinia* spp.). For products that are sufficiently heated prior to consumption, histamine is the main risk. Changing consumer or operator behaviour can further increase the risk, if the temperature range corresponding to this behaviour has not been sufficiently tested.

Control measures
The risk can be minimised by imposing the same requirements on the TTI as those imposed on use by/best before dates. In addition, for both TTIs and best before/use by dates, it is important to monitor trends in consumer behaviour, and food production, to ensure shelf life and to raise awareness of the importance of following instructions for both consumers and operators where necessary.

Conclusion

- If an evaluation of the practical trial shows that instructions are clear, are followed and consumer and operator behaviour when using the TTI does not lead to consumption of unsafe food, the wider use of the TTI alongside use by/best before dates, or as a potential replacement for use by/best before dates, can be considered.
- If TTIs are enshrined in law, the associated risks are similar to those associated with use by/best before dates.
- If TTIs are not sufficiently enshrined in law, a risk to food safety is particularly likely for foodstuffs to be stored refrigerated that contain pathogenic microorganisms that are able to grow during refrigeration. In the case of food products that are consumed insufficiently heated, *Listeria monocytogenes* and *Yersinia* spp. are most likely to pose a risk, whereby it should be noted that there is no legal standard for *Yersinia* spp. The main risk associated with foodstuffs that require heating prior to consumption is histamine.
- The food business operator that attributes the shelf life to the packaging is held responsible for the safety of the food during the indicated sensory shelf life or safe consumption period. This also applies when extending the use of TTIs to other food products and when the recipe for a food product has changed or the operator's behaviour within the cold chain has changed.
- One point to consider is that the insight gained into consumer behaviour during the pilot is a snapshot: consumer behaviour in relation to TTIs needs to be monitored, to ensure that it is possible to intervene in the event of misunderstandings or dangerous developments.

EFSA survey

Given that the Keep-it operator states that this TTI is on the market in Norway, the Norwegian contact was approached personally about their experience with this TTI. The Norwegian Scientific Committee for Food and Environment states that it has not been involved in the authorisation of Keep-it. The Norwegian FSA is up to date with the situation, however, and states that it has made the operator aware of Regulation 1169/2011, and that if the provisions of this Regulation are observed, the indicator may be used.

A survey was also conducted via the EFSA focal point among 38 European countries to determine whether TTIs had already been validated or were in use in these countries. There were 14 (37%) responses (Appendix 2). Of these 14 countries, 13 indicated that they had no experience with testing TTIs in general, or specifically with Keep-It. They also stated that they had no ongoing practical trials with TTIs.

Only Germany reported a practical trial with a freshness indicator app, in which a dynamic best before date is calculated on the basis of input from storage and transport conditions of food products using algorithms (https://www.lebensmittelwertschaetzen.de/aktivitaeten/freshindex/, www.tsenso.com). According to the information provided, this trial does not involve a TTI that is actually affixed to the food products. Studies are under way and this 'fresh index' app has not yet been introduced into use in Germany.

General conclusion

To ensure food safety during a practical trial, TTIs must be introduced with caution:

Safety of TTIs as an instrument
Where a TTI is applied to food products, the danger is that the components, and the quantities used, are unsafe. A risk occurs if these unsafe substances come into contact with the foodstuff or the consumer. Regulation 450/2009 sets out requirements for the marketing of products including TTIs. EFSA has drawn up guidelines in this area (EFSA, 2009). An instrument applied to food products must not contain substances classified as 'mutagenic', 'carcinogenic' or 'toxic for reproduction', and the substances must be safe at the quantities used, or the barrier adequate. In addition, the measures taken to minimise the probability of the functional barrier being breached should be known. The risk is therefore highly dependent on the properties of a specific TTI. In general, the risk of an unsafe TTI is assessed as low based on probability (low) and impact (substance-dependent and dose-dependent; generally mild). For children, this risk is estimated to be moderate, based on probability (low) and impact (limit reached earlier in children; moderate).

Additional risk can arise where a TTI is included in the recycling process for plastic packaging to be reused for food contact materials. If this occurs, substances that are not on the positive list of Regulation (EU) 10/2011 may end up in plastic food contact materials, albeit in a diluted form. To assess the associated risk, it is necessary to know the substances and quantities involved, and how these substances react to processing. In the long term, it is also important to know to what extent these substances can be stacked.

Specific to Keep-it:
The Keep-it indicator is affixed to the outside of the product packaging and comes with a functional barrier to prevent the consumer and the food from coming into contact with the active substances. This means that, under normal circumstances, this indicator does not pose a hazard and therefore a risk. The documentation provided by the Ministry of Health, Welfare and Sport contains a Keep-it hazard analysis, in which one of the critical points states that the Keep-it indicator can be damaged and the indicator fluid can spill out of the indicator. The patent also takes into account deliberate removal of the indicator, which can damage it. If the fluid from the Keep-it indicator comes into contact with food or the consumer, a risk cannot be excluded. In order to assess this risk, it is necessary to know the active substances and the quantities at which they are present in the indicator. The active substances do not feature on the positive list of food contact materials. Where packaging bearing a Keep-it indicator is recycled into food contact materials, these substances may end up in food contact materials in a diluted form. In order to assess the associated risk, it is also necessary to know the quantities of the substances present in the indicator.

Suitability of TTIs as an indicator of sensory shelf life or safe consumption period
One hazard when using a TTI as an indicator of the sensory shelf life or safe consumption period of food products is that the progression of the TTI may not adequately reflect the dynamics of the microbial hazards in the foodstuff in question. A hazard is particularly likely with foodstuffs that are chilled in relation to the ambient temperature. Temperature is a critical factor when it comes to shelf life, particularly with the aim of restricting microbial growth. From a food safety point of view, an incorrect *longer* indication of the safe consumption period (i.e. longer than the use by date) is particularly dangerous. In these foods, refrigeration is very important to limit the growth of pathogens. The risk of a TTI leading to harmful foodstuffs in the event of insufficient correlation with microbial growth is estimated to be reasonable. The risk increases the less conditions a TTI is tested under (extreme temperatures of freezing and heat wave, observed refrigerator temperature at the consumer's home). The risk of this subsequently

leading to illness increases the closer the consumer consumes the food towards the end of the incorrectly indicated sensory shelf life or safe consumption period.

A risk is most likely in the case of *L. monocytogenes* and *Yersinia* spp., which can grow at the recommended refrigerator temperature of 4°C, to some extent histamine *(Proteus*), which can be formed at the recommended maximum refrigerator temperature of 7°C, and to a lesser extent *B. cereus, S. aureus* and *Vibrio* spp., which are formed, grow or produce toxins at a temperature higher than the recommended maximum temperature of 7°C . Although a foodstuff that contains Salmonella or STEC is already harmful at the time of packaging, a longer indicated shelf life increases the risk as the expected refrigerator temperature may allow some growth, and a higher dose can cause a more serious illness.

An additional hazard associated with the use of a TTI as an indicator of the sensory shelf life or safe consumption period of food products is that the progression of the TTI may not be adequately calibrated to the specific food product. The probability of this occurring is higher where the TTI is not affixed during production but later on in the chain and, from that point on, is not adequately calibrated to the specific conditions. The relevant pathogens depend on the properties of a food product. The food business operator is familiar with these properties and is therefore responsible for determining the product's shelf life. Food product suppliers and operators should ensure that foodstuffs meet the legal microbiological criteria during this stipulated shelf life. In addition, they should take into account, on the basis of shelf life studies, any other pathogens relevant to the foodstuff. The relevant pathogens will need to be identified for each foodstuff, or on a 'case by case' basis as also stated by EFSA (EFSA, 2020). The risk of a TTI affixed at a later stage, or a TTI with a preset default expiry time (such as 10 days at 4°C) failing to correlate sufficiently with microbial growth relevant to the food and resulting in a harmful food product is estimated to be moderate.

Specific to Keep-it:
Based on the information provided by the Ministry of Health, Welfare and Sport, the risk of the use of Keep-it currently leading to unsafe fresh chicken meat or unsafe fresh fish/fresh fish fillet is estimated to be moderate, based on probability (moderate) and impact (moderate).

Control measures
A TTI that has been found to be safe can offer significant added value by providing information about the temperature history at individual food product level. Controlling the risks associated with a TTI can have a positive impact on combating food waste while ensuring food safety. EFSA has developed a science-based decision tree for date marking with a use by or best before date(EFSA, 2020). BuRO also considers this decision tree applicable to controlling the risks of an authorised TTI, as the TTI can be viewed as a 'flexible date marker'. The decision tree focuses on an acceptable level of microorganisms during the shelf life (for spoilage microorganisms) or safe consumption period (for pathogenic microorganisms) in relation to food properties. Where the expiry of an authorised TTI for a food product has been determined by following this decision tree, including carrying out the necessary studies, the risk associated with a TTI is assessed as low. As EFSA also points out, this decision tree must be followed for every food product.

Appendix 3a contains the EFSA decision tree, and Appendix 3b features the same decision tree with the addition by BuRO of the times at which supplementary measures can be taken to ensure food safety when using a TTI. BuRO makes a

distinction here between four categories of food, each with its own level of relevance to the TTI, namely:

I. frozen food (best before date)

In these foods, no microbiological growth takes place under normal conditions (i.e. frozen). A TTI can indicate whether the freezing chain has been broken, giving microorganisms the opportunity to grow. Adding an indicator that has been found to be safe as an instrument does not pose a risk to food safety, and may in fact promote food safety.
For TTI mechanisms that continue during freezing, it can be investigated whether the TTI is also suitable for indicating chemical or physical spoilage. Foods that are sold frozen are given a best before date, whereby chemical spoilage is the main determining factor. This type of spoilage generally takes a long time, so the added value of a TTI is limited.

II. food that can be stored at ambient temperatures (best before date)

In the case of these foods, the ability of a TTI to monitor extreme temperatures can be demonstrated. Foods that can be stored at ambient temperatures are given a best before date, which means that a loss of quality can be expected after this date. This loss of quality is, in principle, not pathogenic and is also apparent to the consumer, meaning it is likely that all or part of the food will not be consumed. Temperature is only one of the factors that influence quality loss. A TTI only measures temperature, and can therefore only provide the consumer with limited additional information, namely in relation to factors influenced by temperature (and not those influenced by aspects such as light or moisture). The use of a TTI alongside the best before date on the label can be useful in extreme weather conditions. In this case, the addition of an indicator that has been found to be safe as an instrument does not pose a risk to food safety.

III. food to be stored in a refrigerator due to loss of quality (best before date)

Under foreseeable storage conditions, no pathogen growth or toxin production will occur in these foods. The storage conditions are aimed at controlling the growth of spoilage flora, which is assumed to occur earlier than the growth of pathogens. For these foods, a TTI can be assessed as an indicator of the relevant spoilage flora for the food in question, whereby it should be noted that the relevant spoilage flora may not be limited to those to which a legal criterion applies, but may also include other spoilage flora. The addition of an instrument that has been found to be safe does not pose a risk to food safety if it is affixed by the food operator who is familiar with the properties of the food product and has adjusted the progression and expiry of the TTI to the dynamics of the microorganisms that are relevant to this product.

IV. food in which pathogen growth or toxin production can be expected under foreseeable storage conditions (use by date).

For these foods, a TTI can be assessed as an indicator of relevant pathogenic microorganisms, although it should be noted that the relevant pathogenic microorganisms may not be limited to those to which a legal criterion applies, but may also include other pathogenic microorganisms. The addition of an instrument that has been found to be safe does not pose a risk to food safety, provided that the progression and expiry of the TTI within the expected temperature ranges reflect the expected dynamics of pathogenic microorganisms. It is therefore important that the operator, who is familiar with the properties of the food product, has adjusted the progression and expiry of the TTI to the product.

Practical trial - expected conditions of use in the cold chain

In a practical trial, consumers will actually be exposed to food products bearing a TTI. One hazard is that safety will not be guaranteed during a practical trial. This can occur if the TTI is not authorised for use in food products in accordance with directives, or if the food business operator has failed to adequately demonstrate that the proposed food product will remain safe upon expiry of the TTI under the foreseeable storage conditions. These hazards become risks at the point at which a consumer actually uses the TTI during the practical trial. An additional hazard is that the legal aspects of a TTI are complex. As already stated by the Ministry of Health, Welfare and Sport, an exemption will be required from the compulsory text accompanying a use by date (Section 15, paragraph 3 of the Preparation and Handling of Food [Commodities Act] Decree); in addition, it is not clear whether it is temporarily legally possible to permit the consumption of a food product with an expired use by date, and to what extent a TTI could lead to confusion among consumers.

The practical trial that the Ministry of Health, Welfare and Sport has been asked to approve concerns the sale of six own brand products (three fresh chicken products and three fresh fish products) bearing a Keep-it indicator at 130 stores over a period of 12 weeks. BuRO is not aware of any documentation showing that the Keep-it indicator is authorised for use on food products according to the directives. This is therefore a hazard that could become a risk in a practical trial, although it is worth noting that Keep-it is already being used on food products in Norway. The study design of the practical trial does not state at what point the TTI is affixed to the food, i.e. whether this occurs during production or later in the supermarket. The supermarket chain states that both suppliers have carried out microbiological testing. BuRO has received the results of these tests and assesses that, on that basis, the TTI is still insufficiently calibrated to the selected food products, with a possible risk to food safety. The findings of the microbiological tests show that the TTI has expired at the time the food exceeds the microbiological criteria, however the tests need to demonstrate that the food remains below these criteria just before the TTI expires. At present, it is not sufficiently guaranteed that the food is safe when the Keep-it TTI expires. Only the results for two of the six food products are known to BuRO. This is therefore a hazard that becomes a risk in a practical trial. Keep-it has been tested for consumer understanding and the conclusion reached is that the TTI would not be confusing. However, these results are based on consumer self-reporting and have not been verified, which means that it has not been established whether the consumer has indeed interpreted the TTI correctly.

If a practical trial of the use of a TTI is permitted, a hazard during the practical trial is that the TTI will provoke different behaviour within the cold chain. For example, if temperature-related storage instructions or recommendations are adhered to less strictly due to confidence in the performance of the TTI. Consumers may also find the use of a TTI alongside a use by or best before date confusing. Both situations can lead to the consumption of a harmful foodstuff. BuRO estimates the risk of harmful foodstuffs during the practical trial to be low based on probability (low) and impact (mild) for the average consumer; as moderate based on probability (moderate) and impact (mild) for the food waste-conscious consumer; and as moderate based on probability (low) and impact (moderate) for vulnerable populations. The additional risk compared to the use by/best before date will be limited to the situation where a food is consumed after the use by/best before date. A practical trial is therefore particularly important to gain further insight into consumer and operator behaviour, and consumer understanding, based on the assumption that the TTI has been authorised and is accurate.

Wider use of TTIs

If a TTI meets the above conditions based on the evaluation of the practical trial and the TTI-food combination is also found to be safe in practice, this TTI-food combination can be considered for authorisation, and the extension of use of the TTI to other foods can be considered. In the short term, the TTI will be used alongside a use by or best before date; in the long term, it is conceivable that the TTI will replace a use by or best before date. For both situations, the danger is that the TTI is not sufficiently enshrined in law to oblige and hold food business operators accountable for the necessary studies. In the event that the TTI meets the conditions to be found safe, and is enshrined in law, the risk of harmful foodstuffs is estimated to be low based on probability (low) and impact (mild). In the event that the TTI is not enshrined in law, the risk of harmful foods is estimated to be moderate based on probability (moderate) and impact (mild in healthy people, severe in vulnerable populations). Consumers who are focused on preventing food waste may be at a higher risk if they frequently purchase foods that are on or close to the use by date.

One point to consider is that the insight gained into consumer behaviour during the practical trial is a snapshot. Changing behaviour of both consumers and operators can increase the risk of unsafe food. It is essential to monitor behaviour in relation to TTIs to ensure it is possible to intervene in the event of misunderstandings or dangerous developments.

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Appendix 1: Scientific literature search strategy

The starting point was information from regulations and BuRO products:

- Regulations (EU) No. 1169/2011 and No. 2073/2005
- The BuRO opinion on best before dates (BuRO, 2016)
- risk assessments of the fish, crustacean and shellfish chain (BuRO, 2020a), poultry meat chain (BuRO, 2018a), egg chain (BuRO, 2018b), red meat chain (BuRO, 2015) and dairy chain (BuRO, 2017)

In addition, a scientific literature search was carried out on 3 June 2020 to support the four steps of the risk assessment according to the following search strategy:

- in PubMed using the terms i) [time range time-temperature indicator food]; ii) ['time-temperature' indicator food]; and iii) [food waste shelf life].
- in the EFSA Journal using the terms: iv) [time-temperature indicator in title]; and v) on the basis of the article thus found in the category 'food ingredients and packaging' within the subject 'food ingredients and packaging'.

The articles found were assessed for relevance, namely focusing on TTI on packaging in combination with smart packaging to gain further insight into shelf life and correlation of the TTI with microbiological food safety. Based on relevant articles, search terms in Pubmed were refined as follows:

- vi) [freshness indicator food package]

With the aid of the search terms [time range time-temperature indicator food], Pubmed was used to find out for which time range time-temperature indicators are being developed. These search terms yielded 36 references, of which three were found to be relevant (Vaikousi et al., 2008; Anbukarasu et al., 2017; Choi et al., 2020).

Finally, Pubmed, EFSA Journal and google scholar were searched for literature that explicitly mentions Keep-it (vii).

If a search yielded more than 50 articles, the selection was limited to reviews. Where the full text was not available to BuRO, the risk assessment was based on the information provided in the article summary.

A total of 19 articles were initially selected for further analysis in the literature review. The search terms in i) yielded 36 results, of which 3 were found to be relevant (Vaikousi et al., 2008; Anbukarasu et al., 2017; Choi et al., 2020); ii) yielded 197 results, which were narrowed down to 13 reviews, of which 5 were relevant (Cutter, 2002; Ozdemir & Floros, 2004; Buncic et al., 2014; Realini & Marcos, 2014; Sohail et al., 2018); iii) 16 results of which 3 were relevant (Corradini, 2018; Mustafa & Andreescu, 2018; Liegeard & Manning, 2020); (iv) 1 result that was also found to be relevant (EFSA, 2013); (v) 31 results of which 2 additionally relevant (EFSA, 2009;2016a); *(vi) 18 results of which 3 relevant (Lee & Shin, 2019; Yimenu et al., 2019; Wu et al., 2020); (vii) yielded 2 relevant results (Skjervold et al., 2007; Koutsoumanis & Gougouli, 2015).*

The following literature has been added further to a BuRO internal review and assessment round:
Additional search shelf life spoilage instead of pathogens: (Food Safety Authority of Ireland; PrimeSafe; Food Science Australia, 2002; Kaur et al., 2017; Possamai et al., 2018; Gratta et al., 2019; Tsironi et al., 2019; Mouafo et al., 2020)
(National Research Council, 1985; Koutsoumanis, 2001; Terpstra et al., 2005; Antoniewski & Barringer, 2010; DTU Food, 2014).

A risk assessment was also specifically carried out for Keep-it, focusing on the active substances.

Appendix 2: Survey on experience with TTIs and Keep-it via EFSA in 38 European countries

Expert consultation on the use of time-temperature indicators

The Dutch Ministry of Economic Affairs is looking for ways to reduce food washed in the context of food waste policy. An option currently considered is the use of a time-temperature indicator (TTI) as an voluntary addition to the legally obliged 'use by' date for fresh products, as is regulated in Regulation (EU) 1169/2011 chapter V, article 36. Appendix X of this regulation states the following:

2. The 'use by' date shall be indicated as follows:
(a) it shall be preceded by the words 'use by ...';
(b) the words in point (a) shall be accompanied by:
- either the date itself, or,
- a reference to where the date is given on the labelling,
Those particulars shall be followed by a description of the storage conditions which must be observed;

A Dutch supermarket chain is asking permission to test a time temperature indicator (TTI) for fresh products like chicken and fish, that need to be stored between 0 and 7 degrees of Celsius. The indicator will be used in a practical setting. The exact indicator used that is asked permission for is 'Keep-it', and based on a chemical substance of which the contents are not known to us. If stored at low temperatures throughout the transport chain, the shelf life of fresh products may be prolonged with several days compared to the 'use by' date. Thereby the TTI could contribute to reducing food waste. However, for the accompanying text an exemption could be considered for this pilot, provided that the use of TTI ensures food safety.

The Office of Risk Assessment & Research (BuRO) of the Dutch Food and Consumer Product Safety Authority (NVWA) was requested to advise on the food safety. Although the permission was asked for a pilot in a practical setting, with one specific TTI brand to be applied on two specified products (i.e. chicken and fish), our interest goes to TTI's in general (i.e. irrespective of the brand), to find wether or not usage of TTI's corresponds with food safety.

Our questions to the member state representatives:

	No	Yes
1 Do you in your country have experience with *testing* time-temperature indicators?	14	0
2 Do you know of a test running in your country to test a time-temperature indicator *in a practical setting* like a supermarket?	13	1
3 Do you in your country have experience with *the use* of time-temperature indicators and in particular Keep-it, in a practical setting for fresh products?	14	0

If one of the answers to the above questions is 'yes', please answer the additional questions on the following page.

If you have experience with testing or using TTIs:

	No	Yes	If yes, link to protocol
4 Do you have a protocol for allowing TTIs in your country?	1		

	No	Yes
5 Which TTI is tested or in use in your country?		
- Keep-it		
- Other, i.e.		1

	No	Yes	If yes, describe products
6 In which products did you investigate correlation with microbiological food safety?	1		
- fresh products of animal origin			
- ready-to-eat products			
- frozen products			
- other			

	No	Yes	If yes, product tested:	If yes, microorganism species tested
7 Have you checked for correlation of the TTI with microbiological food safety, i.e. with respect to growth of:	1			
- bacteria				
- yeast				

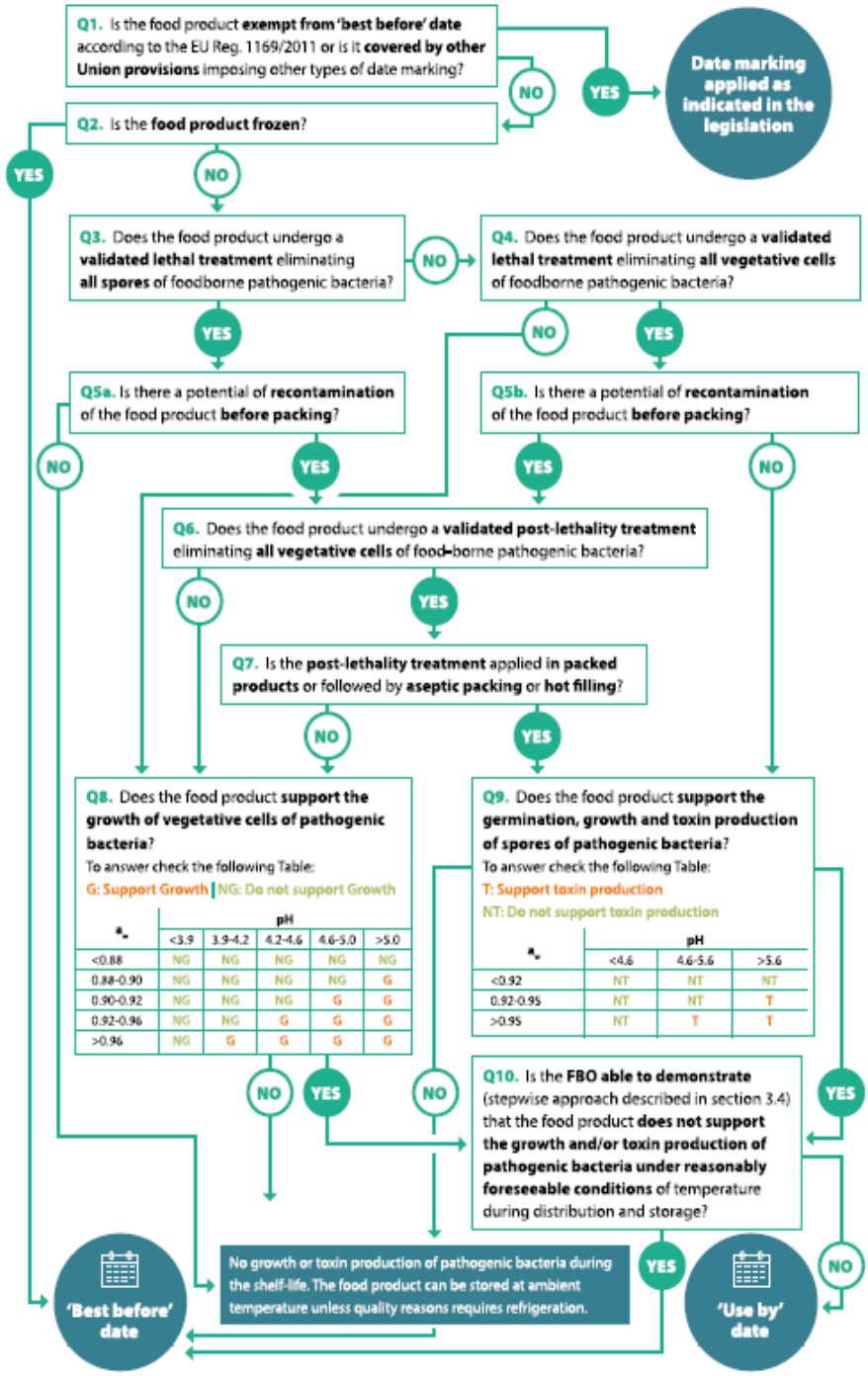
	No	Yes	Temp (°C)	Duration (hours, days, weeks)
8 If tested, which scenario's did you consider?				
- Temperatures as applied in food chain		1		
- Transport in summer/winter				
- Consumer use - shopping on warm day				
- Consumer use - keeping in freezer				
- Recipe changes (f.e. less salt)				
- Other, ...				

9 Have you checked for chemical food safety with use of TTI?	Specify chemicals considered
- potential of leakage of substance	
- potential of migration of substance	
- Other, i.e.	

	No	Yes	In addition to 'use by' date	Instead of 'use by' date
10 In which settings are TTIs currently used in your country?	1			
- In hospitals				
- In supermarkets				
- In horeca				
- Other, ...				

	No, we don't have	No, they are confidential	Yes, attached to e-mail	Yes, link provided:
11 Is it possible to share reports or risk assessments on this topic?				1

Appendix 3a: EFSA decision tree on date marking (best before date/use by date) (EFSA, 2020).



a_w	pH <3.9	3.9-4.2	4.2-4.6	4.6-5.0	>5.0
<0.88	NG	NG	NG	NG	NG
0.88-0.90	NG	NG	NG	NG	G
0.90-0.92	NG	NG	NG	G	G
0.92-0.96	NG	NG	G	G	G
>0.96	NG	G	G	G	G

a_w	pH <4.6	4.6-5.6	>5.6
<0.92	NT	NT	NT
0.92-0.95	NT	NT	T
>0.95	NT	T	T

Figure 1: Decision tree on the appropriate date marking for temperature controlled prepacked foods

Appendix 3b: EFSA decision tree on date marking (best before date/use by date) (EFSA, 2020) with options identified by BuRO for the addition of TTIs (see key).

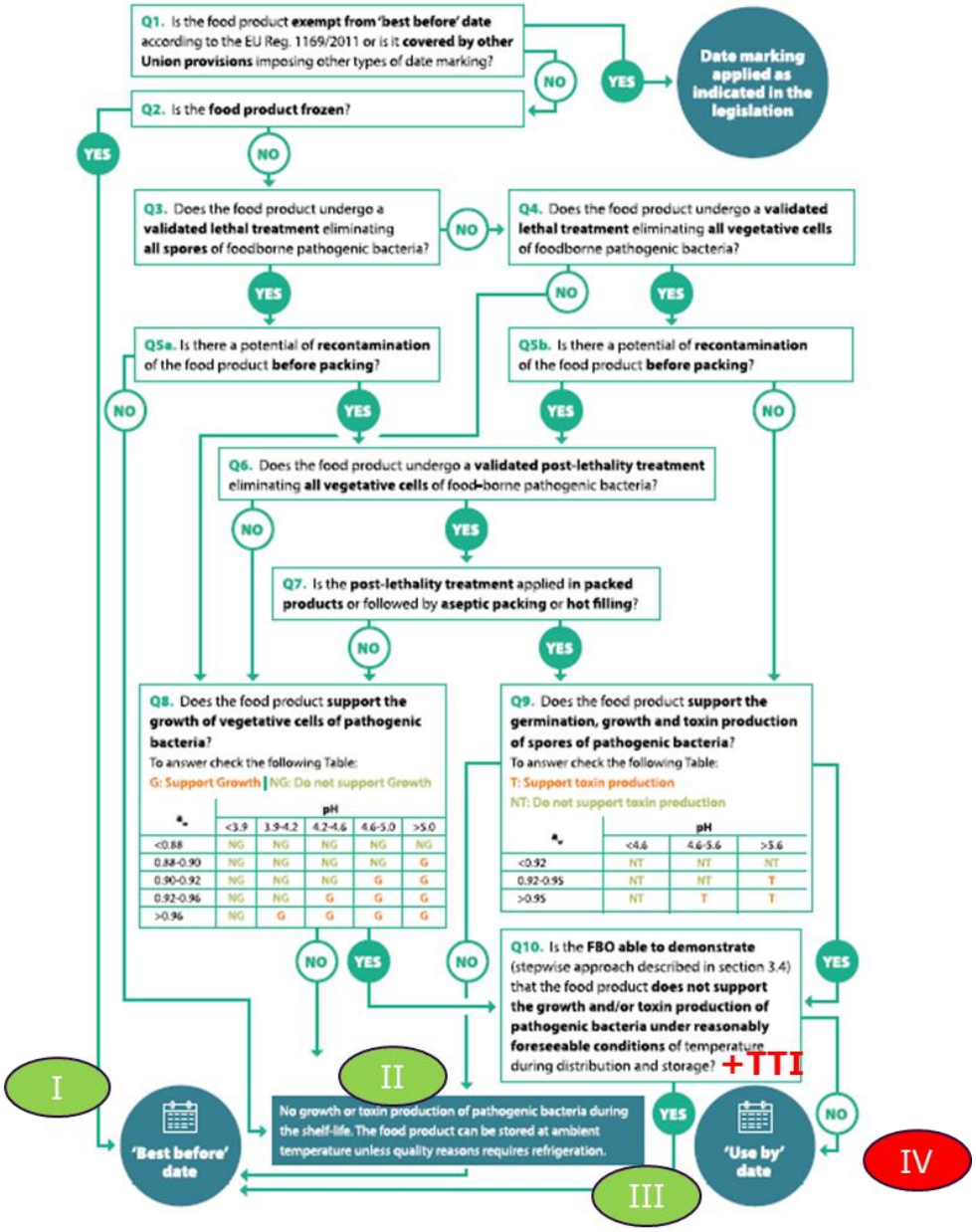


Figure 1: Decision tree on the appropriate date marking for temperature controlled prepacked foods

Key to BuRO additions:
+TTI: Include TTI as one of the foreseeable conditions in Q10
I: Does the TTI indicate an interruption of the freezing chain?
II: Does the TTI indicate quality loss related to extreme temperatures?
III: Does the TTI correlate with relevant spoilage microorganisms?
IV: Does the TTI correlate with relevant pathogenic microorganisms?

= no risk to food safety; risk to food safety

Appendix 4: Comprehensive hazard characterisation of pathogenic microorganisms in refrigerated foodstuffs

For the properties per pathogenic microorganism and their toxins (including biogenic amines and histamine), BuRO's chain risk assessments use data from the US Food and Drug Administration (Food Drug Administration, 2012), as included in the most recent risk assessments (BuRO, 2020b;2020a).

Salmonella
Salmonella has a minimum growth temperature of 5.2°C, although not for all serotypes. However, *Salmonella* does not need to grow to be pathogenic, with a theoretical infectious dose of one cell (Food Drug Administration, 2012). This means that a foodstuff containing *Salmonella* is already unsafe at the time it is packaged. The standard is set at absent in 25 grams. However, the risk of illness increases with the ingestion of larger numbers of *Salmonella* cells. Due to the relatively low growth temperature of *Salmonella*, growth, where present in the food, is possible at various realistic time-temperature storage profiles. *Salmonella* causes typhoid fever, paratyphoid fever and salmonellosis. In 2018, more than 14,000 DALYs in the Dutch population were attributed to *Salmonella* in food. The main route is through foodstuffs from pigs, poultry and cattle. It is not known what proportion of this was refrigerated food.

Enterobacteriaceae
Besides *Salmonella*, there are a couple of other relevant hazards belonging to the *Enterobacteriaceae* family: *Shigella* and *Yersinia* spp. Standards apply to carcasses after slaughter but before refrigeration. However, growth can occur after refrigeration. *Shigella* has a minimum growth temperature of 6.1°C and *Yersinia* spp. of -1.3°C. *Shigella* requires little growth (10–200 cells) to be pathogenic. A *Shigella* infection can be asymptomatic, mild (dysentery) or severe (HUS). Vulnerable populations (children, the elderly) are more likely to have a serious course of infection. It is estimated that 100 to 1,000 people in the Netherlands become infected with *Shigella* through food every year. The proportion of these infections that can be attributed to refrigerated food is not known. *Yersinia* does require growth to be pathogenic, with an infectious dose of $\geq 10^4$ to 10^8 cells. The disease caused by *Yersinia* spp. is called yersiniosis. The symptoms are mild (gastroenteritis, reactive arthritis). Although there is no recent data, it was estimated that 900–10,000 cases of *Yersinia* disease were foodborne in the Netherlands in the period 1991–1996. These infections are generally associated with pork food products. It is not known what proportion is attributable to refrigerated food.

Vibrio spp.
Vibrio spp. has a minimum growth temperature of 10°C. The infectious dose varies between *Vibrio* spp. ranging from $\geq 10^3$ cells to 10^6 cells (Food Drug Administration, 2012). *Vibrio* spp. can cause cholera or vibriosis. Cholera does not occur in the Netherlands. The symptoms of vibriosis are mild (gastroenteritis) to severe (sepsis). No specific criteria have been set for *Vibrio* spp. in food. The risk assessment of the fish, shellfish and crustacean chain estimates that *Vibrio* spp. infections are almost non-existent in the Netherlands, however there is also no obligation to report them. This estimate may be an underestimate.

S. aureus
S. aureus has a minimum growth temperature of 7°C. Production of the pathogenic toxin occurs from a growth temperature of 10°C and a concentration of $>10^5$ cfu/g. *S. aureus* therefore requires growth to be pathogenic. *S. aureus* toxin-mediated disease is characterised by nausea, vomiting and abdominal cramps with or without diarrhoea. Vulnerable populations (children, the elderly)

are more likely to have a serious course of infection. The limit is 100,000 cfu/g (Food Drug Administration, 2012). In 2018, more than 250,000 DALYs were attributed to *S. aureus* toxin in food in the Netherlands (Appendix 6). Most cases involve prepared food with unhygienic hand contact, but also cheese. It is not known what proportion involved packaged refrigerated food. The storage temperature required for growth and toxin formation over a longer period of time is not likely for packaged foods.

B. cereus
B. cereus has a minimum growth temperature of 4°C. The bacterium requires growth to become pathogenic. The infectious dose is 10^5 cfu/g, and ingestion of cells or spores can be pathogenic. No toxin formation takes place below 10°C (Food Drug Administration, 2012). The limit is 100,000 cfu/g. Symptoms of illness caused by *B. cereus* are mild (gastroenteritis). In 2018, more than 47,000 DALYs were attributed to *B. cereus* toxin in food in the Netherlands. Most cases involve prepared food. It is not known what proportion involved packaged refrigerated food. The storage temperature required for growth is not likely for packaged foods.

Histamine
Histamine is formed by enzymatic reactions, by enzymes derived from bacteria *(Proteus*). Storing fresh fish in a refrigerator (0–4°C) minimises histamine formation, as the bacteria involved cannot grow properly and the enzymes involved are not activated. Histamine is not destroyed when fish is cooked or fried. In EU Member States, fishery products must not contain more than 200–400 mg of histamine/kg. In the Rapid Alert system for Food and Feed, 12 reports of excessive histamine levels in fish were made between 1 January 2020 and 20 September 2020. Foods with a histamine concentration of more than 50 mg per 100 g of fish (500 mg/kg) can cause histamine poisoning (Feng et al., 2016; BuRO, 2020a). The symptoms resemble an allergic reaction and are often mild and short-lived, which is why people do not seek help and why it is unknown how many cases per year there are in the Netherlands. Outbreaks are regularly reported in Europe, such as three outbreaks in Sweden caused by tuna from Vietnam (Whitworth, 2020). In the Netherlands, three outbreaks were reported in 2016 and two in 2017 (Friesema et al., 2018). The storage temperature required for growth in packaged foods is likely to occur.

L. monocytogenes
L. monocytogenes has a minimum growth temperature of -0.4°C. Listeria requires some growth to become pathogenic. The infectious dose is 10^3–10^4 cells (Food Drug Administration, 2012). For *L. monocytogenes*, the standard for ready-to-eat foods is 100 cfu/g for the duration of the shelf life, and absent in 25 g at the time of production. In most people, *L. monocytogenes* infections cause no symptoms or only mild ones (such as gastroenteritis); severe symptoms caused by *L. monocytogenes* include encephalitis and meningitis, sepsis, miscarriage and stillbirth. Vulnerable groups are most likely to have a serious course of infection. In 2018, just over 50 DALYs were attributed to *L. monocytogenes* in food in the Netherlands. Most cases involve ready-to-eat foods (such as meat products and smoked fish) and, in particular, packaged refrigerated foods. The storage temperature required for growth is very likely to occur for packaged foodstuffs. Heating prior to consumption can kill the bacteria and thus stop it from causing disease.

STEC/EHEC
In the case of pathogenic *E. coli*, such as STEC, a minimum growth temperature of 6.5°C and an infectious dose of 10 to 100 cells apply (Food Drug Administration, 2012). STEC therefore requires little growth to be pathogenic. This means that a

foodstuff containing STEC is already unsafe at the time of packaging. The standard is set at absent in 25g (sprouts). Although STEC requires little or no growth to be pathogenic, the probability of illness increases with consumption of larger quantities. Due to the growth temperature of STEC, growth, where present in the food, is possible at realistic time-temperature storage profiles. Disease caused by STEC ranges from mild (such as gastroenteritis and haemorrhagic colitis), to severe (e.g. haemolytic uremic syndrome [HUS]) to chronic (e.g. End Stage Renal Disease). In 2018, approximately 850 DALYs were attributed to STEC in food in the Netherlands. The main route is through foodstuffs from cattle. It is not known what proportion of this was refrigerated food.

C. botulinum
C. botulinum is strictly anaerobic, has a minimum growth temperature of 3°C and requires growth and toxin formation to be pathogenic. It causes very serious illness. The toxin is heat labile and is inactivated when cooked. Cases are often associated with fermented foods. *C. botulinum* causes botulism. Cases rarely occur in the Netherlands (BuRO, 2020a). Refrigeration is essential to control the hazard.

Burden of disease (based on current situation with use by dates)
The burden of disease from microbiological and chemical/enzymatic hazards stemmed from various food products in 2018. Burden of disease in DALY is a combination of probability and impact. This risk assessment briefly touches on this subject in order to outline the current food safety situation, as the currently known burden of disease is based on the impact as influenced by the currently applicable use by and best before date. For a more detailed description of the effects of various pathogenic microorganisms, please refer to BuRO's chain risk assessments. The currently known burden of disease in the Netherlands can be broken down as follows into the chains and pathogenic microorganisms described here, as based on the RIVM 2018 disease burden estimates, whereby no distinction is made for refrigerated food (Pijnacker, 2019):

Table 4: disease burden estimates in DALYs in the Netherlands, 2018, attributed to food products

	Beef and lamb	Pork	Poultry	Eggs	Dairy	Fish and shellfish
Salmonella	1,800	2,100	2,100	3,200	950	590
STEC/EHEC	380	55	27	18	63	25
B. cereus toxin	3,400	1,700	760	1,700	2,700	950
S. aureus toxin	19,000	20,000	20,000	8,300	37,000	15,000
L. monocytogenes	6	5	4	2	13	10
Vibrio spp.*	-	-	-	-	-	-
Histamine*	-	-	-	-	-	-

* No disease burden estimates are available for *Vibrio* spp. and histamine.

Appendix 5: Comprehensive hazard characterisation for Keep-it components

Potassium ferrocyanide ($K_4[Fe(CN)_6]$)
Potassium ferrocyanide, following oral intake of 10 mg, is absorbed by the gastrointestinal tract of rats to a limited extent. Most was excreted unchanged via faeces. Ferrocyanide was found in the liver, spleen, kidneys, heart and lungs. Unbound iron was found in erythrocytes (red blood cells) and unbound cyanide was found in urine and exhaled air. In humans, absorption following oral exposure is low (0.25–0.42%) (EFSA, 2018).

Potassium ferrocyanide has a low acute oral toxicity. Following oral intake of potassium ferrocyanide by rats, an LD_{50} between 1600 and 5,110 mg/kg of body weight was reported (EFSA, 2018; ECHA, 2020). Following dermal exposure in rats, an LD_{50} of 2,000 mg/kg of body weight was reported (ECHA, 2020). When it comes to the use of ferrocyanide as a food additive, EFSA has no concerns regarding genotoxicity. Ferrocyanide is not carcinogenic. There is no data on reproductive toxicity (EFSA, 2018). Ferrocyanide does not irritate or sensitise the skin (ECHA, 2020).

EFSA has derived an acceptable daily intake (ADI)[8] for sodium, potassium and calcium ferrocyanide of 0.03 mg of ferrocyanide ion/kg of body weight per day. The ADI is based on a two-year study that looked at renal toxicity. A 'no observed adverse effect level' (NOAEL) of 4.4 mg of sodium ferricyanide/kg of body weight per day was derived from this study. Taking into account a safety factor of 100, this leads to an ADI of 0.044 mg of sodium ferrocyanide/kg of body weight per day. EFSA assumes that the toxicity is caused by the ferrocyanide ion and has therefore derived a group ADI for all three substances (expressed as ferrocyanide ion) (EFSA, 2018).

The REACH registration dossier states 'a derived no-effect level' (DNEL)[9] for dermal exposure for the general population of 4.5 mg/kg of body weight per day (ECHA, 2020).

Iron

The element iron in the body is a component of haemoglobin (in red blood cells), myoglobin (in muscle tissue) and a number of enzymes. The body needs iron in order to function optimally. Iron deficiency anaemia is treated by means of supplementation. Iron is only absorbed as the dissolved divalent (ferrous) form. The dissolved iron enters the mucosal cell where oxidation to the trivalent (ferric) form takes place (Greupink R. et al., 2015).

EFSA derived dietary reference values[10] for iron in 2015. The average requirement for men and post-menopausal women is 6 mg/day. For premenopausal women, the population reference intake (PRI) is 16 mg/day. For children, PRI varies according to age: 11 mg/day (7–11 months), 7 mg/day (1–6 years), 11 mg/day (7–11 years and boys aged 12–17 years). For girls aged 12–17 years, the PRI is 13 mg/day (EFSA, 2015).

Toxic effects can be expected at doses from 20 mg/kg of elemental iron. Ingestion of more than 60 mg/kg is considered a severe case of poisoning (Greupink R. et al., 2015).

Potassium

Potassium is a cation that plays a vital role in the acid-base balance, isotonicity and electrodynamic properties of the cell. It is essential in many enzymatic reactions and in numerous physiological processes, such as muscle contraction, transmission of action potentials, protein synthesis and carbohydrate metabolism. The potassium balance in the body is precisely regulated by absorption from food in the intestine and excretion through the kidneys (Nielen J.T.H. & Vanmolkot F., 2016).

[8] An ADI is an estimate of the amount of a substance that individuals are able to take in daily, for the rest of their lives, without experiencing any significant health effects.
[9] A DNEL is the level of exposure to the substance that should not be exceeded in humans.
[10] Dietary reference values or recommended daily allowances (RDAs) are a measure of how much of a nutrient healthy people need on a daily basis.

EFSA derived dietary reference values for potassium in 2016. EFSA suggests an adequate intake (AI) of 3,500 mg/day for adults. An AI of 750 mg (7–11 months) is suggested for babies. For children, the AI ranges from 800 mg (1–3 year olds) to 3,500 mg (15–17 year olds). An AI of 4,000 is suggested for breastfeeding women because these women excrete potassium through breast milk (EFSA, 2016b).

The toxic dose is highly dependent on kidney function, as with normal kidney function the body is able to eliminate excess potassium quickly. Gastrointestinal side effects are mainly associated with local effects (Nielen J.T.H. & Vanmolkot F., 2016).