**SCIENTIFIC COMMITTEE
OF THE FEDERAL AGENCY FOR
THE SAFETY OF THE FOOD
CHAIN**

Superior Health Council

<u>Common Advice</u>
<u>SciCom 14-2014 and SHC Nr. 9160</u>

<u>Subject:</u> Food safety aspects of insects intended for human consumption (Sci Com dossier 2014/04; SHC dossier n° 9160)

Advice approved by the Scientific Committee of the FASFC on 12 September 2014 and validated by the Board of the Superior Health Council (SHC) on 3 September 2014

Summary

In the search for alternative dietary protein sources, insects appear to offer great potential. Currently there are no specific regulations neither in Belgium, nor in Europe, on the breeding and marketing of insects destined for human consumption. The trade of a number of insect species destined for human consumption is however tolerated in Belgium. In this context, the Scientific Committee and the Superior Health Council are asked to give advice on the potential risks (hazards) associated with the consumption of these insects (entomophagy).

Worldwide there are about 2.000 edible insects species known and, in certain regions, insects are already eaten for ages by humans. Nevertheless, there is only little scientific literature available on the food safety of insects. To guarantee the food safety of entomophagy on a large scale, more research on the microbial and chemical safety of insects destined for human consumption is needed.

In this advisory report, the potential microbial, chemical (including allergens) and physical hazards specifically related to the consumption of insects are discussed. These hazards depend on the insect species, the cultivation conditions (feed and environment) and the subsequent processing, and can largely be controlled by the adequate application of the prevailing good hygiene and manufacturing practices during breeding and marketing of insects. Nevertheless, a heating step before consumption is indispensable as well as the mentioning of appropriate storage and preparation conditions on the label. The label should additionally contain a warning for a possible allergic reaction of persons allergic to seafood and/or dust mites.

Keywords

Keywords	Mesh terms*	Sleutelwoorden	Mots clés	Stichwörter
Nutrition	**“Diet”**	Voeding	Nutrition	Ernährung
Risk assessment	**“Risk assessment”**	Risico-evaluatie	Evaluation du risque	Risiko-Bewertung
Recommendations	**“Nutrition policy”**	Aanbevelingen	Recommandations	Empfehlungen
Food safety	“Food safety”	Voedselveiligheid	Sécurité alimentaire	
Insects	“Insects”	Insecten	Insectes	
Entomophagy		Entomofagie	Entomophagie	Entomophagie

** MeSH (Medical Subject Headings) is the NLM controlled vocabulary thesaurus used for indexing articles for PubMed.*

List of abbreviations

cfu	colony forming units
EC	European Commission
FASFC	Federal Agency for the Safety of the Food Chain
GHP	Good Hygienic Practices
GMP	Good Manufacturing Practices
HAAs	heterocyclic aromatic amines
HACCP	hazard analysis and critical control points
MPN	most probable number
FHFS	Food and Health, Food Safety included
OTA	ochratoxin A
PAHs	polyaromatic hydrocarbons
SHC	Superior Health Council
TSE	transmissible spongiform encephalopathies

1. Reference terms

1.1. Question

The Scientific Committee of the FASFC (Federal Agency for the Safety of the Food Chain) and the Superior Health Council (SHC) are asked if human consumption of insects presents a risk for public health, or in other words, which microbiological, chemical, physical and parasitological risks (hazards) are associated with the consumption of insects.

The question only pertains to:
- insects intended for human consumption;
- insects that are consumed as a whole or as a 'preparation of the whole insect' (e.g. grinding of whole worms);
- insects that are cultivated in a standardized environment;
- the twelve insect species listed in **table 1**, which correspond with the species that are most frequently offered for human consumption on the Belgian market (in 2011)[1].

The advice does not go into the food safety of:
- insects that are bred for feed;
- the human consumption of fractions of insects (protein preparations or other extracts obtained from insects);
- insects harvested in the wild.

Table 1. Insects offered for human consumption on the Belgian market (in 2011) and which are discussed in the present advice[*]

Latin name	**English name**	**Stage of development at the time of consumption[**]**
Acheta domesticus	house cricket	adult (imago)
Achroia grisella	lesser wax moth > wax moth worm	caterpillar
Alphitobius diaperinus	litter beetle[***] > lesser mealworm	larva
Alphitobius laevigatua	buffalo worm > lesser mealworm	larva
Bombyx mori	Silkmoth > silkworm	pupa (without cocoon) & caterpillar
Galleria mellonella	greater wax moth > waxworm	caterpillar
Gryllodes sigillatus	banded cricket	adult (imago)
Gryllus assimilis	field cricket	adult (imago)
Locusta migratoria	African migratory locust	larva & adult (nymph & imago)
Schistocerca americana	American desert locust	adult
Tenebrio molitor	yellow meal beetle > yellow mealworm	larva
Zophobas atratus	morio beetle -> morio worm (Eng.: 'superworm')	larva

[*] With the exception of *Alphitobius laevigatua* and *Gryllus assimilis*, marketing these insects is tolerated in Belgium pending European legislation.
[**] Note however, that it is sometimes difficult to make a distinction between the larvae of the *Alphitobius laevigatua* and the *Alphitobius diaperinus* and between the nymphs of the *Gryllus assimilus* and the *Gryllodes sigillatus*.
[***] Next to lesser mealworm, the larva of the litter beetle is often (wrongly) referred to as buffalo worm (NVWA, 2012).

1.2. Legal context

- Regulation (EC) N° 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety

[1] This table was drawn upfollowing a survey by the European Commission (EC) in member states to gather information on insects that are offered for human consumption and are put on the market in Europe.

- Regulation (EC) N° 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs

- Regulation (EC) N° 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients

- Regulation (EC) N° 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene

Regulation (EC) N° 258/97 stipulates that food or food ingredients which have not been used before 15 May 1997 for human consumption to a significant degree within the European Union are novel foods or novel food ingredients. Under this Regulation, a risk assessment shall be undertaken for all novel foods or novel food ingredients and an authorization shall be delivered by the European Commission (EC) before they are allowed to be placed on the market. This authorization concerns the conditions of use, the designation of the novel food or novel food ingredient and the specific labelling requirements.

Currently, there is legal uncertainty as to whether whole insects or preparations thereof (e.g. worm paste) fall within or outside of the scope of Regulation (EC) N° 258/97. The EC has, however, recently drawn up a new proposal for revision of this Regulation. According to this draft, all species and forms of insects are considered 'novel food', unless it can be shown that they were consumed to a significant extent by humans in the European Union before May 15th 1997. This new Regulation still has to be approved by the European Parliament and the Council of the European Union. At the time of publication of the present advice, there was no clarity yet on the planned timing of publication.

While awaiting this harmonization of the European legislation, the trade of some insects is tolerated on the national territory (see **table 1**, with the exception of *Alphitobius laevigatua* and *Gryllus assimilus*). This tolerance does, however, not apply to ingredients that were isolated or extracted from insects, such as protein isolates (see also statement FASFC [2], and FPS Health, Food Chain Safety and Environment [3]).

1.3. Methodology

After analyzing the advisory requests to the SHC and the Scientific Committee of the FASFC, it was decided to provide a common advice.
The advice is based on a review of the relevant[4] scientific literature, published in scientific journals and reports from national and international organisations with competencies in the field (peer reviewed), as well as on the opinion of experts.

Considering the discussions in the meetings of the common work group held on 21 February and 12 May 2014 and in the plenary session of the FASFC's Scientific Committee on 17 January, 21 March, 20 June and 12 September 2014, the meeting of the permanent work group 'Food and Health, Food Safety included' (FHFS) of the SHC on 22 January, 26 February and 27 August 2014 and of the Board of the SHC of 3 September 2014,

[2] http://www.favv.be/foodstuffs/insects/
[3] http://www.gezondheid.belgie.be/eportal/foodsafety/19091496_EN?fodnlang=en#.U9ZN16O_H19
[4] The available data from literature are scarce, and are generally based on single experiences, with the provided information limited to the experimental protocol.

the Scientific Committee and the Superior Health Council provide the following common advice

2. Introduction

Considering the problems that occur in the production of animal proteins concerning the environment (climate, environmental hygiene, biodiversity), the world food issue (food supply, animal production efficiency, third world problems), excessive consumption, etc., alternative sources of food proteins are becoming increasingly important. Examples of possible alternative forms of protein production are 'novel protein foods' (based on vegetable protein carriers and based on micro-organisms), cultured meat, algae and insects (van der Spiegel *et al*., 2013; Cazaux *et al.*, 2010).

Worldwide, about 1,500 to 2,000 insect species are considered fit for human consumption, among which *Coleoptera* (beetles), *Lepidoptera* (butterflies and moths), *Hymenoptera* (bees, wasps and ants), *Orthoptera* (locusts and crickets), *Isoptera* (termites), *Hemiptera* (half-winged) and *Homoptera* (cicadas).[5] Human consumption of insects, also known as entomophagy, as an important source of proteins is prevalent in many cultures spread all over the world. Even though eating arthropods, such as lobsters, is common in Western Europe and they are considered a delicacy, eating insects is rather uncommon and is often considered strange. Nevertheless, insects are also processed into food in certain European regions. Examples of this are the cockchaver (May beetle) soup eaten in France and Germany, the local tradition in the North Italian region of Carnia of eating the sweet-tasting crop of *Zygaena* day moths (and the similar Syntomis) and the casu marzu, a Sardinian cheese containing larvae of the cheese fly. **Table 1** lists which insects are most frequently offered for human consumption on the Belgian market.

Edible insects, also referred to as 'micro livestock' or 'mini livestock', are quite nutritious. They contain proteins (with a composition similar to meat), vitamins, minerals and fatty acids; the specific nutritional value and the chemical composition is determined by the species, the stage of development and the feed (Belluco *et al.*, 2013; FAO, 2013; van Huis, 2013; Siemianowska *et al.*, 2013; Verkerk *et al.*, 2007; Finke, 2002).
Depending on the species, insects are consumed by humans in different stages of development, namely in the egg, larva, pupa or adult stage (Belluco *et al.*, 2013; Verkerk *et al.*, 2007; Finke, 2002). Insects are mostly consumed in their entirety, but they can also be processed into pastes or powders. The extraction of proteins, fats, chitin, minerals and vitamins from insects is also possible (FAO, 2013).

3. Advice

3.1. <u>Production of insects intended for human consumption</u>

The processes that are used for farming insects may differ significantly from one another and information on this is scarce. Moreover, the specific cultivation conditions such as the amount of light/lighting, air humidity, ventilation, population-/ larvae density, the oviposition site, the availability of water and feed (e.g. chicken feed, certain vegetables or waste streams), do not only depend on the cultivated species but also on the desired development stage of the insect at the time of consumption (Rumpold & Schlüter, 2013). After being harvested, the insects can be sold raw, but also dried, ground, pulverized, heated (steamed, cooked, roasted,

[5] An extensive list is provided among others on the following web pages:
- http://www.foodinsectsnewsletter.org/pdfs/Worldwidespecieslist15sept2011.pdf
- http://www.wageningenur.nl/en/Expertise-Services/Chair-groups/Plant-Sciences/Laboratory-of-Entomology/Edible-insects/Worldwide-species-list.htm

baked, fried), canned or freeze-dried. There are several ways of preparing and consuming insects, but they are often consumed in their entirety without removal of the intestinal tract. In controlled cultivation, the insects are (often) not fed for a while before being harvested, so they can empty their bowel contents (NVWA, 2012).

A schematic overview of the production process is displayed in **figure 1.**

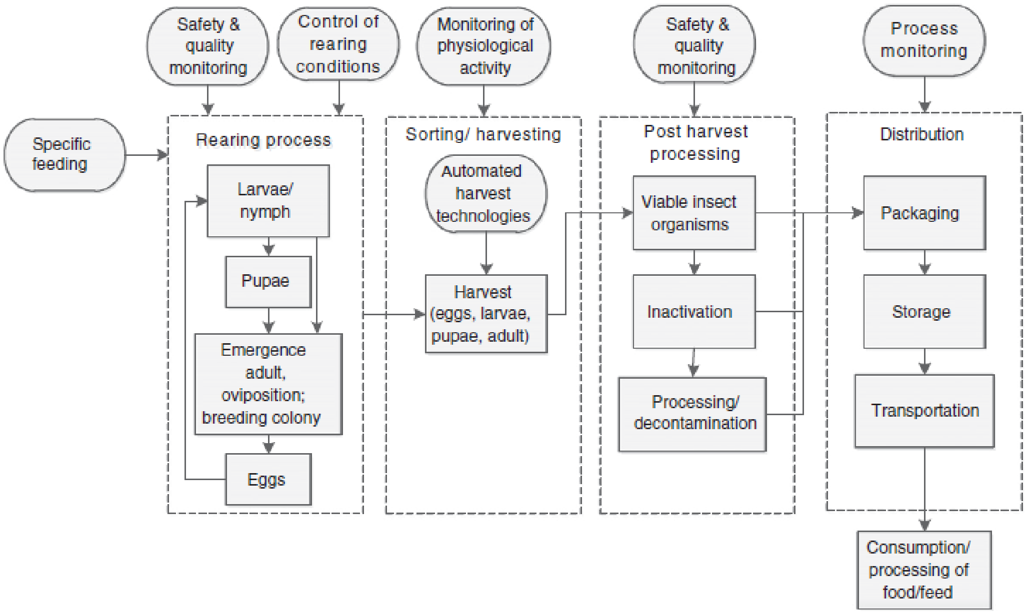

Figure 1. Schematic representation of the production process of food and feed based on edible insects (source: Rumpold & Schlüter, 2013).

For the purpose of illustration, a few 'industrial' cultivation processes of a number of relevant insects are described below.

- Mealworms and lesser mealworms (*Tenebrio molitor*, *Alphitobius diaperinus*) are cultivated on a growth medium consisting of bran with meal or ground chicken feed, complemented with carrots, potatoes and water. After the beetles (i.e. adult insects) have laid their eggs, it takes about 8 to 10 weeks (at a temperature of 28-30 °C and 60% relative humidity) before the larvae can be harvested. The larvae are sieved out of the growth medium and are put in a cooler (6-15 °C) without feed for a couple of days so that they can empty their intestinal contents. Subsequently, the larvae are rinsed clean in lukewarm water and are frozen at a temperature of -18 °C. After being frozen, the larvae are freeze-dried (NVWA, 2012).

- Migratory locusts (*Locusta migratoria*) lay their eggs in a substrate of peat. After about 10 days, the young hatch and are transferred into production bins (20-25 °C). They are fed on dry grass and bran and can be harvested after 26 to 28 days. After 2 days of feed deprivation, the locusts are frozen and freeze-dried (NVWA, 2012).

- Silk worms (*Bombyx mori*) are mostly fed on the leaves of the white mulberry. After an incubation period of 7 to 10 days at 15 to 20 °C the larvae hatch. They further develop at a temperature of 25 °C and 75-80% relative humidity and go through 4 moults (i.e. 5 stadia, 26-30 days) after which they are harvested for human consumption (Harizanis, 2007).

3.2. Hazards associated with the consumption of insects

Just like vertebrates, insects can contain biological agents and substances, which can represent a health threat when consumed. Cultivation, processing and further storage conditions largely determine the food safety of edible insects. For instance, special attention should go to the bacterial, mycological and toxicological risks associated with the consumption of insects that were fed on manure and related organic waste streams, and the risks will be greater for insects that were harvested in the wild than for those that are cultivated in a standardized environment (NVWA, 2012; FAO, 2013).

As previously mentioned (paragraph 1.1.), this advice only pertains to the risks related to the consumption of whole insects or 'preparations of whole insects' (e.g. grinding of entire worms), and not to the consumption of the protein preparations or other extracts of these insects. Only the hazards associated with insects that were cultivated in a standardized environment will be discussed, not those associated with insects that were harvested in the wild. Although insects are also cultivated for animal feed, the advice only pertains to the use of insects in human food.

In what follows, the potential hazards related to the consumption of the insects mentioned in **table 1**, are described. However, information available in the literature is scarce, and is mostly based on single experiments with limited information on the experimental design.

3.2.1. Microbial hazards

Both insects harvested in the wild and cultivated insects have a large diversity of micro-organisms in their intestinal flora and spores of different micro-organisms may be present on the insects' cuticula or exoskeleton (FAO, 2013; NVWA, 2012).

There are few specific scientific studies on the microbiological safety of edible insects that are cultivated under controlled conditions. In the available studies a fairly high bacterial count between 10^5 en 10^7 cfu/g is reported.

In a study on fresh, cultivated morio worms (*Zophobas morio*), yellow mealworms (*Tenebrio molitor*), waxmoth worms (*Galleria melonella*) and house crickets (*Acheta domesticus*) the microbiological flora mostly consisted of Gram-negative bacteria, among which faecal and total coliform bacteria. The Gram-positive population mostly consisted of *Micrococcus spp*., *Lactobacillus spp*. (10^5 cfu/g) and *Staphylococcus spp.* (about 10^3 cfu/g). Neither *Salmonella* nor *Listeria monocytogenes* were found (Giaccone, 2005 cited in Belluco *et al*., 2013). Another study revealed that in fresh, cultivated meal worms (*Tenebrio molitor*) and house crickets (*Acheta domesticus*) 10^4-10^6 cfu/g *Enterobacteriaceae* and 10^2 - 10^4 spore-producing bacteria were found, which usually do not belong to a pathogenic species (Klunder *et al.*, 2012). Similar high values of 10^7 cfu/g for the total aerobic bacterial count, but also for the total anaerobic bacterial count and *Enterobacteriaceae* were measured in a preliminary Belgian study on mealworms (*Tenebrio molitor*), locusts (*Locusta migratoria*) and morio worms (*Zophobas atratus*). Lower values of < 10 cfu/g for *Enterobacteriaceae* were measured on raw silk worms (*Bombyx mori*) (internal communication 13/05/2014, F. Wouters & R. Binst, VIVES - association K.U.Leuven [6]). In another exploratory Belgian study on raw and frozen mealworms (*Tenebrio molitor*) and locusts (*Locusta migratoria*) similar high values (10^7 - 10^9) for the aerobic bacterial count and aerobic spores in the order of 10^4 cfu/g were measured (internal communication 02/05/2014, J. Stoops & L. Van Campenhout, Lab4Food, K.U.Leuven).
The number of bacteria that are present in the intestinal tract of insects, varies from 10^8 to 10^{11} per ml of intestinal contents and mainly consists of Gram-negative rod bacteria and

[6] results exploratory research concerning edible insects in the framework of the GROWTH project, spring, 2010

Gram-positive cocci (Cazemier, 1999). *Escherichia coli*, *Enterobacter liquefaciens*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pantoea* (= *Enterobacter*) *agglomerans,* among others, and also a number of Gram-positive cocci were isolated from the intestinal tract of cultivated locusts (*Schistocerca gregaria*) (Dillon & Charnley, 2002). Some insects (flies, beetles to a lesser extent) are described in the literature as vectors of *Salmonella spp.* and *Campylobacte*r *spp.* in livestock and poultry (Belluco *et al.,* 2013*;* Wales *et al*., 2010).

Moreover, epizootic infections may occur in the farming of insects, which decimates the insect harvest. The house cricket (*Acheta domesticus*) is, for instance, sensitive to the densovirus AdD-NV) (Szelei *et al.*, 2011) and *Enterococcus mundtii* was identified as one of the agents in the disease "flacherie" in silkworms (*Bombyx mori*) (Cappellozza *et al.*, 2011). Because insects are taxonomically much more distant from humans than "conventional livestock", there is reason to assume that the risk of zoonotic infections is low (but not inexistent) (FAO, 2013). The risk of zoonotic infections (but also of microbial contamination in general) increases in case of careless use of waste materials, unhygienic treatment of the insects, and direct contact between cultivated insects and insects from outside of the company. The literature for instance mentions cases of botulism, parasitosis and food poisoning (e.g. by aflatoxins) that are related to entomophagy (Schabel, 2010).

Important factors that influence the microbial safety of insects, namely the nutrient medium and the farming environment (3.2.1.1.), the processing procedures that are applied (3.2.1.2.) and the storage conditions (3.2.1.3.) are discussed in greater detail below.

3.2.1.1. Nutrient media & farming environment

The intestinal flora of insects can contain a great diversity of parasites, fungi and other micro-organisms, in which the farming environment as well as the nutrient media play an important role. Some insects (mainly flies, but also to a lesser extent beetles) are, for instance, described as vectors of *Salmonella* and *Campylobacter* in livestock and chickens (Belluco *et al*., 2013; Wales *et al*., 2010), and larger numbers of spore-producing bacteria were found in house crickets (*Acheta domesticus*) that are cultivated in containers filled with soil than in mealworm larvae (*Tenebrio molitor*), which are cultivated in wheat flour (Klunder *et al.*, 2012).

Since insects' intestinal microflora is a reflection of the cultivation environment, the intestinal flora of insects of the same species may differ. In addition, the intestinal flora could be influenced by the insect's nutritional status. Dillon & Charnley (2002) report a larger population[7] of bacteria in starved insects than in insects that are fed (Dillon & Charnley, 2002). However, more information about the effect of emptying the intestinal contents by fasting (as is often applied in the controlled cultivation of insects) on the population of intestinal micro-flora is needed in order to set off the microbial risk of consuming these insects against the risk of consuming insects with remaining intestinal contents.

Furthermore, in the context of potential microbiological risks, it might be useful to verify whether it is possible to positively influence the intestinal flora of insects by adding certain ingredients to the feed (as, for example, observed in broiler chicks in which the consumption of prebiotics curbs the colonization of enteropathogens like *Clostridium spp*. and *Salmonella spp*.). It needs to be noted, however, that for some insect species changes in the composition of the feed did not seem to cause changes in the intestinal flora (Rumpold & Schlüter, 2013; Colman *et al*., 2012; Andert *et al.*, 2010).

Pathogenic fungi, such as *Aspergillus*, *Penicillium, Mucor* and *Rhizopus* can infect host insects via feed. These fungi can be directly contagious for humans or can give off secondary substances that are toxic or allergenic. *Aspergillus*, *Penicillium* and *Fusarium* fungi are for instance often associated with the production of toxic mycotoxins (FAO, 2013; NVWA, 2012;

[7] In the publication (Dillon & Charnley, 2002) it is not specified whether the 'larger' population pertains to a larger number or to a larger diversity of insects.

Schabel, 2010) (also see 3.2.2.2.). In a preliminary Belgian study yeasts and fungi were found in considerable amounts in fresh, freeze-dried as well as in frozen mealworms (*Tenebrio molitor*) and locusts (*Locusta migratoria*) (internal communication 02/05/2014, J. Stoops & L. Van Campenhout, Lab4Food, K.U.Leuven).
In order to prevent the formation of fungi, it is strongly recommended to work, if possible, with dry cultivation soils and to regularly remove the faeces and/or to refresh the feed. It is also recommended to disinfect the cultivation room, the breeding beds and the material after each cultivation cycle.

Transmissible spongiform encephalopathy (TSE) agents could pose a risk. Studies have shown that insects fed on elements of the nervous system of ruminants infected with the scrapie agent, were themselves a source of contamination (Lupi, 2006 & 2003; Post *et al*., 1999; Rubenstein *et al*;, 1998; Wisniewski *et al*., 1996).
If insect feed contains animal products, a risk assessment should be conducted regarding the transmission of TSE-agents.

3.2.1.2. Processing procedures

- Thermal treatment

In an exploratory study, oven drying (110 min. at 90 °C) of meal worms (*Tenebrio molitor*) and locusts (*Locusta migratoria*) seemed to reduce the total aerobic bacterial count (> 3,0 x 10^7 cfu/g) by 2 to 3 log (to 1,2 x 10^5 and 3,5 x 10^4 respectively) and the amount of *Enterobacteriacea* (> 1,5 x 10^7 cfu/g) by 3 to 5 log (to 2,5 x 10^4 and 2,2 x 10^2 respectively). Boiling (8 min. at 100 °C) reduced the total aerobic bacterial count and the amount of *Enterobacteriacea* to < 10 cfu/g (internal communication 13/05/2014, Wouters F. & Binst, R., VIVES, association K.U.Leuven). Another study that used mealworm larvae (*Tenebrio Molitor*) and house crickets (*Acheta domesticus*) also showed that boiling (5-10 min., at 100 °C) strongly reduces the amount of *Enterobacteriacae* (from 10^7 in mealworm larvae and 10^4 cfu/g in house crickets to < 10 cfu/g), but roasting (10 min., no temperature mentioned) did not (a reduction to 10^2 - 10^3 cfu/g in mealworm larvae). Blanching before roasting did result in a strong reduction of the number of *Enterobacteriacae*. The spores however appeared not to be fully inactivated by roasting nor by cooking (Klunder *et al.*, 2012). In addition, the surviving spores can be brought to germination by the processing procedures and grow during storage. Spore-producing bacteria are consequently a potential risk associated with entomophagy. A thermal treatment like sterilization and appropriate storage conditions (see 3.2.1.3) are consequently strongly recommended.

Another possible hazard related to the consumption of raw or insufficiently heated insects, is the fact that the consumer may catch a parasitic infection. That is to say, parasites can use insects as a(n) (intermediate) host or as a 'temporary' host (Belluco *et al.*, 2013; NVWA, 2012; Chai *et al.*, 2009; Hinz, 2001). A number of gastro-intestinal worms (helmints) are documented which can be found in, among other things, human excrements and for which insects specifically serve as intermediate hosts (Hinz, 2001).

- Freeze-drying

Cultivated insects are usually not heated during the production process, but are sometimes frozen or freeze-dried. When freeze-drying, water is extracted from the product at freezing temperatures to obtain a relatively long shelf-life (when stored in a cool and dry place)[8]. In a preliminary study freezing as well as freeze-drying barely seemed to influence the microbial quality (total aerobic bacterial count) of the insects (internal communication 02/05/2014, J. Stoops & L. Van Campenhout, Lab4Food, K.U.Leuven). In a microbial analysis of 55 freeze-dried insect products (locusts, lesser mealworms, mealworms and mealworm snacks) from

[8] Freeze-drying may, however, be accompanied by an undesired oxidation of the unsaturated long-chain fatty acids, which causes a reduction of the product's nutritional value.

the Dutch retail business (in 2010), the amount of aerobic bacteria turned out to be higher than 10^6 cfu/g in 59% of the samples and in 65% of the samples more than 10^3 cfu *Enterobacteriaceae* per gram was found. *Clostridium perfringens*, *Salmonella* and *Vibrio* were not found and in 93% of the samples the concentration of *Bacillus cereus* was lower than 100 cfu/g (NVWA, 2012). These values of the aerobic bacterial count and the concentrations of *Enterobacteriaceae* and spore-producing bacteria in the freeze-dried mealworms (*Tenebrio Molitor*) are comparable to the values Klunder *et al.* (2012) mention in the case of fresh mealworms.

- Grinding

Grinding of (raw) mealworm larvae (*Tenebrio molitor*) could increase the number of bacteria in a homogenized sample[9], probably because of the release of microbiotics from the intestine (with the possibility of further growth, depending on the storage temperature and storage duration). Grinding of insects before frying or cooking would not improve the efficacy of the thermal treatments, but on the contrary would lead to a higher microbial load compared to whole mealworm larvae (Klunder *et al.*, 2012).

- Lactic fermentation

Ground insects can also potentially be used in fermented food in order to increase the protein content. The acid medium created by fermentation increases the shelf-life and microbial safety of (ground) insects.
This was illustrated by Klunder *et al.* (2012) using a mixture of roasted mealworm larvae (10-20%, *Tenebrio molitor*), water and flour. Lactic fermentation of this mixture stabilized the population of spore-producing bacteria (< 10^3 cfu/g). In addition, the acidified conditions (pH 3,7), inhibited the germination and the growth of the spores.

- Manipulation

As is the case for other foodstuffs, microbial contamination can take place when further handling the insects. In the literature a contamination with *Staphylococcus sp.* of thermally treated larvae is documented which, in addition to a wrongly applied thermal treatment, can also be attributed to the larvae being handled by healthy carriers of *Staphylococcus sp.* (Rumpold & Schlüter, 2013).

3.2.1.3. Storage conditions

As already mentioned above (3.2.1.2.), the presence of spore-producing bacteria in which the spores may survive further processing (e.g. heat treatment below sterilization conditions, like roasting, etc.) and in which further processing can also induce the germination of these spores, constitutes an important, potential hazard (e.g. for botulism) in cultivated insects. In addition to a thermal treatment, appropriate storage conditions are consequently also important (Schabel, 2010). Under certain circumstances (e.g. temperatures around 30 °C and a damp environment) the germinated spores can further develop and as such lead to food spoilage. In an exploratory study spore-producing bacteria were found in the intestines and the cuticula of cultivated mealworm larvae (*Tenebrio Molitor*) and house crickets (*Acheta domesticus*). These bacteria were mainly *Bacillus licheniformis*, and sporadically *B. subtilis* and *B. megaterium*. These *Bacillus* species are often found in the soil and should not be pathogenic, but they could lead to food spoilage (Klunder *et al.*, 2012).

There are no conclusive scientific studies that verify the microbial safety of insects during or after the shelf-life (e.g. 52 weeks) mentioned by the manufacturers. In the framework of the preliminary tests of the shelf-life of mealworm paste (without additives), the total aerobic

[9] Evidence for an increase was found by Klunder *et al.* (2012), but wasn't observed by J. Stoops & L. Van Campenhout (internal communication 02/05/2014, preliminary study, Lab4Food, K.U.Leuven).

bacterial count, the *Enterobacteriaceae*, the total anaerobic bacterial count, yeasts and fungi were analysed at various moments under various storage conditions. The results of this study suggest an indicative shelf-life between 3 and 7 days at 2-7 °C when the paste is kept in a sterile jar; when stored under vacuum atmosphere the paste can be stored for 7 days, and after pasteurization of the paste in a sterile jar, it can be stored for more than 14 days (internal communication 29/04/2014; F. Wouters, VIVES - association K.U.Leuven).

3.2.2. Chemical hazards

There are mainly two sources for the presence of toxic substances in insects, namely the production of natural toxins by some insects at a certain stage of development and the intake of contaminants or phytochemicals via feed (van der Spiegel *et al.*, 2013).

3.2.2.1. Natural toxins

Some insects naturally contain repellent or toxic chemical substances, e.g. as a part of their defensive mechanism (Dzerefos *et al*., 2013; Rumpold & Schlüter, 2013). The defensive secretions of mealworms *(Tenebrio molitor*) for example, contain quinones that could be toxic, carcinogenic and mutagenic. However, as far is known only the adult beetles, and not the larvae that are consumed, contain or give off quinones (NVWA, 2012). As a result, the development stage is important for the consumption of some insects[10]. In addition, it is noted that, even though certain insect species look strongly alike, one species is safe to consume, while the other isn't or is only edible under certain circumstances.

Other examples of defensive secretions that can be reactive, irritating or toxic, are among others carbon acids, alcohols, aldehydes, alkaloids, ketones, esters, lactones, phenols hydrocarbons and steroids (van der Spiegel *et al*., 2013). There are no immediate indications that the insects listed in **table 1** and in the instar mentioned at the time of consumption excrete reactive, irritating or toxic substances.

Toxicological tests conducted on whole insects or insect proteins are almost non-existent (NVWA 2012) and up till now there are no risk evaluations available that verify the 'toxic dose' of insects (that are poisonous or that are only to be consumed under certain conditions). Zhou & Han (2006) assessed the safety of (purified) proteins of silkworm pupas (*Antheraea pernyi*)[11] via a number of acute and subacute toxicological tests and showed that these proteins are neither acute toxic, nor genotoxic or mutagenic.

3.2.2.2. Chemical contaminants and residues

A lot of the chemical hazards associated with the consumption of cultivated insects are linked to the habitat or the farming environment and the feed and, consequently, these can be controlled.

As is the case in ‘conventional’ livestock farming, the feed is determining for the (undesired) chemical substances that can accumulate in cultivated insects. Examples of such contaminants are dioxins, PCBs (polychlorinated biphenyl compounds), heavy metals, pesticide residues, but also fungicides and antibiotics that are added to nutrient media to counter microbial contamination (Belluco *et al.*, 2013; Devkota & Schmidt, 2000). As is the

[10] Although the difference between the adult and the larva stages is sufficiently marked in mealworms, this difference is less pronounced in Orthoptera, namely crickets and locusts, which undergo a gradual metamorphosis. They change in small steps, which are called the nymph instar, with amoult at the end of each instar. The nymph instar can be distinguished from one another, for instance based on wing development.
[11] The caterpillars of both the *Bombyx mori* and the *Antheraea pernyi* are used in silk production. The pupas (without cocoon) of both species are consumed.

case in ‘conventional’ livestock farming, antibiotics for example, can for the mass-cultivation of insects be added to the feed to avoid big harvest losses because of bacterial infections. However, information on possible residues and the quantities that can be left in the insect, is almost non-existent. A study on silkworms (*Bombyx mor*i) has shown that chloramphenicol (a broad-spectrum antibiotic, however its use is prohibited in animal production (Regulation (EU) N° 37/2010 [12])), is not deactivated in the intestines of the silkworm, neither during autoclaving (sterilization at a high temperature) (Cappellozza *et al.*, 2011).

Another potential chemical contamination hazard lies in the replacement of high quality feed, such as chicken feed, with organic residual flows. Some insect species, among which the mealworm (*Tenebrio molitor*), are very efficient in bioconverting organic waste, which can offer an interesting avenue for making insect farming more profitable. This practice is, however, accompanied by the risk of heavy metals from the environment accumulating in different parts of the insects - in fat, in the shell (exoskeleton), the reproductive organs and the digestive system. A study has shown an accumulation of cadmium and lead in mealworm larvae (*Tenebrio molitor)* that were fed on organic soil material in which these metals were present (Vijver *et al.*, 2003). However, after each moult the accumulated cadmium should be partially (and a large part after metamorphosis) removed from the larvae, but further research is needed (FAO, 2013).

In addition, insects, depending on the feed, can contain phytochemicals such as phenols, flavins, tannins, terpenes, polyacetylenes, alkaloids, cyanogenes, glucosinolates and mimetic amino acids (van der Spiegel *et al.*, 2013).

As already mentioned under 3.2.1.1., insects can also potentially be contaminated with mycotoxins (e.g. aflatoxins, ochratoxin A (OTA)).

Finally, it needs to be mentioned that also during the further processing of insects, toxic substances or process contaminants, such as heterocyclic aromatic amines (HAAs), polyaromatic hydrocarbons (PAHs), acrylamide, chloropropanols and furans can be formed by chemical reactions between the insects and other ingredients (van der Spiegel *et al.*, 2013). This, however, requires further research .

3.2.3. Allergens

Several insects can cause allergic reactions like eczema, rhinitis, conjunctivitis, angioedema and bronchial asthma. For instance, a lot of caterpillars are routinely cleaned and irritating hairs and other protrusions are burned or removed in any other way so as to avoid irritation and inflammation of the skin, eyes and respiratory tract (lepidopterism), possibly causing dermatitis, algogenic and allergic reactions, and even death (FAO, 2013; Schabel, 2010). The majority of these allergic reactions are caused by inhalation (e.g. dust with cockroach faeces) and contact (e.g. caterpillar hairs) and primarily occurs with people who regularly come into contact with insects (e.g. entomologists, fish bait breeders, etc.) (FAO, 2013; Panzani & Ariano, 2001). The same goes for mealworms (*Tenebrio molitor*) and lesser mealworms (*Alphitobius diaperinus*) (Siracusa *et al.*, 2003; Schroeckenstein *et al.*, 1990 & 1988). Nevertheless, a number of cases are documented in which the consumption of insects caused an allergic reaction and even an anaphylactic shock (FAO, 2013; Ji *et al.*, 2009).

There are indications that people who are allergic to crustaceans and shellfish and/or dust mites could have an allergic reaction to the consumption of insects, and this among other things due to cross-reactivity (Verhoeckx *et al.*, 2014; FAO, 2013; Panzani & Ariano, 2001). People who, for example, have allergic reactions to tropomyosins[13] of crustaceans and shellfish or of dust-mites, could possibly develop a sensitivity to tropomyosins of insects.

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[12] Regulation (EU) N° 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin

[13] These are actin-binding proteins that regulate muscle contractions.

Another insect component, that is also linked to allergenicity, although less frequently, is chitin. Chitin is a naturally occurring polysaccharide of glucosamin which can be found, among others things, in the cell walls of fungi and the exoskeleton of crustaceans (e.g. crabs, lobsters and shrimp) and insects. On the one hand, studies suggest that chitin is an allergen. On the other hand, there are indications that chitin and its derivative chitosan (produced industrially via de-acetylation of chitin) have properties that could increase the immuno-response, depending on the administration route and the size of the chitin particles (FAO, 2013; Muzzarelli, 2010; Lee *et al*., 2008). Based on an EFSA-opinion, which states that the intake of 5 g of chitin-glucan from crustaceans should not raise concerns about public health (EFSA, 2010), it is assumed that a daily intake of a portion of 45 g of freeze-dried insects with a chitin percentage of about 6% does not give rise to concerns about public health (NVWA, 2012).

For most people, however, the consumption of and/or the exposure to insects do not represent a significant risk on an allergic reaction (FAO, 2013). Nevertheless, it is strongly recommended to mention on the label that people who are allergic to crustaceans and shellfish and/or dust mites could have an allergic reaction to eating insects.

3.2.4. Physical hazards

Orthoptera (locusts, crickets) and *Coleoptera* (beetles, cicadas) often have powerful lower jaws, firm legs (with sometimes spines on the tibia or shin bones), wings and other appendages which, unless they are removed before consumption, can perforate the intestines or or can get stuck in the intestines and cause constipation (Schabel, 2010). Therefore, it is strongly recommended, if necessary, to mention on the product label that the legs and wings of the insect have to be removed before consumption.

Indigestible chitin rests as well, can accumulate in different places in the colon and cause constipation (FAO, 2013).

3.3. <u>Possible criteria</u>

There are no specific regulations (microbiological requirements, etc.) for the production and commercialization of insects intended for human consumption. As is the case for other foodstuffs, the requirements of the 'General Food Law' have to be met (Regulation (EC) 178/2002). This law prohibits the marketing of foodstuffs if they can cause harm to the public health or are not suited for human consumption. Producers and distributors of insects are also subject to Regulation (EC) 852/2004 on the hygiene of foodstuffs. This regulation requires, among other things, that food business operators are registered and have a preventive self-checking system based on the HACCP ('hazard analysis and critical control point') principles.

Also on the level of the Codex Alimentarius there are no standards available that are specifically related to the use of insects in human food or animal feed (PROteINSECT report "Deliverable 5.1", September 2013[14]). Similar to other foodstuffs of animal origin, the following norms apply (wholly or partially) to edible insects under the Codex Alimentarius (FAO, 2014):

- General Principles of Food Hygiene (CAC/RCP 1-1969), http://www.codexalimentarius.org/download/standards/23/CXP_001e.pdf
- Hygienic Practices for meat (CAC/RCP 58-2005), http://www.codexalimentarius.org/download/standards/10196/CXP_058e.pdf
- The Codex code of practice on good animal feeding (CAC/RCP 54-2004), http://www.codexalimentarius.org/download/standards/10080/CXP_054e.pdf

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[14] *"Deliverable 5.1" – Mapping Exercise Report with regard to current legislation and regulation: Europe and Africa & China*. http://www.proteinsect.eu/fileadmin/user_upload/deliverables/D5.1t-FINAL.pdf

For possible food safety and process hygiene guideline values, the criteria for 'comparable' products (**table 2**) mentioned in Regulation (EC) N° 2073/2005 of the Commission of November 15 2005 on microbiological criteria for foodstuffs can be used.

Table 2. Possible guideline values based on food safety and process hygiene criteria (Regulation (EC) N° 2073/2005)

food category	micro-organism	threshold values	remark concerning the use of the criterion for edible insects
<u>Food safety criteria</u>			
Ready-to-eat foodstuffs that are able to support the growth of *Listeria monocytogenes*	*L. monocytogenes*	100 cfu/g [1]	
Minced meat and meat preparations made from other species than poultry intended to be eaten after heating	*Salmonella*	absence in 10g [1]	Only provided that it can be guaranteed that the insects will still be heated.
Live bivalve molluscs and live echinoderms, tunicates and gastropods (e.g. snails)	*Salmonella* *E. coli* [2]	Absence in 25g [1] 230 MPN/100g of flesh and intra-valvular liquid [1, 3]	
<u>Process hygiene criteria</u>			
Minced meat	Aerobic colony count *E. coli* [2]	m = 5×10^5 cfu/g M = 5×10^6 cfu/g m = 50 cfu/g M = 500 cfu/g	Based on the (limited) information available in the literature, it is difficult to achieve this criterion for raw insects. Nevertheless, this criterion can be used as a reference value and ought to be attainable in a controlled and hygienically executed process.

(1) Products placed on the market during their shelf-life
(2) *E. coli* as an indicator of faecal contamination
(3) MPN: most probable number
(4) 'm' and 'M': values between which a certain number of units should be taken

4. Conclusions and recommendations

In the literature, there is little information to be found regarding the hazards related to human consumption of farmed insects. The available information is not very detailed and/or relies on the extrapolation of information on the consumption of other foodstuffs. Although there are a number of ongoing projects, more research is needed, among other things, on the effect of processing and the different conservation methods (cooking, steaming, baking, frying, grinding, cooling, freezing, canning, freeze-drying, modified atmosphere packaging) on the microbial and chemical safety of insects. Nevertheless, it seems highly unlikely that insects that were farmed under controlled, hygienic circumstances, would get infected with viral or parasitic pathogens from the farming environment or the nutrient medium. Since it cannot be excluded that pathogenic bacteria (and spores) from the production environment may infect the insects and its consumers, a heating step (minimally blanching, cooking, frying or stir frying) is indispensable before the products are put on to market or consumed.

Contamination with yeasts and fungi that can produce harmful secondary metabolites (mycotoxins) is also to be avoided.

As far as chemical hazards are concerned, composition and possible defensive secretions need to be assessed for each insect species separately. There are however no indications that the insects listed in **table 1** contain or secrete such natural toxins in the stage of consumption. Allergic reactions after consuming *Arthropoda* or arthropods are possible, although information on this is scarce.

Recommendations

For the cultivation, processing, marketing, and storage of insects and their products, the same health and sanitary rules (cf. Good Hygienic Practices (GHP) and Good Manufacturing Practices (GMP)) that apply to other conventional foodstuffs (or feedingstuffs) need to be followed in order to ensure food safety[15]. Because of their biological nature, there a number of more specific issues with regard to the microbial safety and toxicity of insects, including :

- The chemical and microbiological risk potential of edible insects largely depends on the nutrient media that are used. These do not only need to be sufficiently checked in terms of microbiological components (e.g. yeasts, fungi), but also in chemical terms (e.g. residues of pesticides, environmental contaminants, etc.). The production or cultivation of edible insects is an activity within primary production, more specifically of animal production intended for human consumption. The feedingstuffs or the growth media that are used, also need to comply with the regulations on feed [16]. Considering the potential hazards, processed wood, manure, kitchen waste and kitchen leftovers must not be used as feed for the cultivation of insects. It is also strongly advised to refrain from using fungicides and pharmacological substances (e.g. antibiotics) in insect feed, since the residual contents of these substances in insects has not been (sufficiently) established yet. If insect feed contains products of animal origin, a risk assessment should be conducted related to the transmission of agents responsible for transmissible spongiform encephalopathies (TSE).

- Considering the fact that the available results suggest that the microbial quality of 'raw insects' is not acceptable, a treatment that kills off micro-organisms, e.g. thermal treatment (blanching, cooking, frying or stir frying) is essential before consumption.

[15] For informative purposes it is mentioned that a circular has been published on the FASFC's website in order to clarify the currently prevailing regulations concerning the cultivation and placing on the market of insects and foodstuffs based on insects for human consumption (http://www.afsca.be/foodstuffs/insects/_documents/2014-05-21_Circular_insects_version11_EN.pdf).

[16] http://www.favv-afsca.fgov.be/productionanimale/alimentation/produits/

- Moreover, the possibility of contamination after processing should be minimized. Storage conditions that limit the growth of micro-organisms should thus be mentioned on the label.

- It is also strongly recommended to work, if possible, with dry cultivation soils and to regularly remove the faeces and/or to refresh the feed, as well as to disinfect the cultivation room, the breeding beds and the material after each cultivation cycle.

- It is strongly recommended to mention on the label that people that are allergic to crustaceans and shellfish and/or dust mites could have an allergic reaction to the consumption of insects.

- Where applicable, the label should mention that the feet and/or wings have to be removed before consumption (e.g. locusts).

- The consumer is strongly advised not to buy insects for their own consumption from breeders of insects that are intended for fish or bird feed, feed for 'new companion animals', reptiles and other insectivores, for which the cultivation process does not take into account possible hazards in case of human consumption. Insect breeders have to make sure to keep the line intended for human food strictly separated from the line intended for animal feed.

5. Recommendations for research

There are different avenues for research to be developed concerning the subject matter:

- Study about the effect of emptying the intestinal contents by fasting (as is often applied in the controlled cultivation of insects) on the population of intestinal micro-flora is needed in order to set off the microbial risk of consuming these insects against the risk of consuming insects with remaining intestinal contents.

- Study regarding the effect of processing on the (micro)biological quality.

- Study regarding the microbial safety of insects during or after the shelf-life mentioned by the manufacturers.

- Toxicological study: risk evaluation to verify the 'toxic dose' of insects that excrete toxins at the developmenal stage at which they are consumed.

- Study regarding possible chemical contaminants and residual levels left in the insect (dioxins, PCBs, heavy metals, pesticides, fungicides, antibiotics). In this context, it would be useful to set up a study on the elimination of cadmium after each moult.

- Study on the effect of further processing of insects on the formation of toxic substances or process contaminants, such as heterocyclic aromatic amines (HAAs), polyaromatic hydrocarbons (PAHs), acrylamide, chloropropanols and furans.

For the Scientific Committee,
The President,
Prof. Dr. Etienne Thiry,

For the Superior Health Council,
The President,
Prof. Dr. Jean Nève

Brussels, 29/09/2014

Brussels, / /2014

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Composition of the working group

The following experts have cooperated in drawing up the advice in the framework of a common working group Sci Com - SHC:

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The administration was represented by:

K. Bergen	FASFC
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Following persons were heard:

F. Francis (ULg) and E. Haubruge (ULg) were heared during the First meeting of the working Group.

The presidency of the working group was assumed by Marianne Sindic and the scientific administration by Wendie Claeys (SciCom), Michèle Ulens (SHC) and Anouck Witters (SHC).

Approval / Validation

The advice was approved by the FASFC's Scientific Committee during the plenary session of 12 September 2014 and by the permanent working group "Food and Health, including Food Safety" of the SHC during the session of 27 August 2014. It was validated by the Board of the SHC during the session of 3 September 2014. The names of the experts of the SHC appointed by Royal decree as well as the members of the Board and Committee are available on the website of the SHC (link: composition and operation).

The Scientific Committee is composed of the following members (http://www.favv-afsca.fgov.be/comitescientifique/membres.asp):
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Conflict of interest

The experts of the working group have completed a general and an *ad hoc* declaration of interests. The potential risk of a conflict of interest was evaluated by the SHC's Deontological Committee and the Bureau of the Scientific Committee. No conflict of interest was established for the experts of the working group.

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Legal framework of the advice

For the Scientific Committee:

Law of 4 February 2000, on the creation of the Federal Agency for the Safety of the Food Chain, in particular article 8;

The Royal Decree of 19 May 2000, on the composition and operating procedures of the Scientific Committee, as established within the Federal Agency for the Safety of the Food Chain;

The Internal Rules as mentioned in Article 3 of the Royal Decree of 19 May 2000, on the composition and operating procedures of the Scientific Committee, as established within the Federal Agency for the Safety of the Food Chain, approved by the Minister on 9 June 2011.

For the Superior Health Council (SHC):

The Superior Health Council is a federal service that is a part of the FPS Health, Food Chain Safety and Environment. The Council was established in 1849 and provides scientific advice on public health to the ministers of public health and environment, to their administrations and to some of their agencies. It provides this advice on demand or on its own initiative. The SHC does not take policy decisions, nor does it execute these decisions, but based on the most recent scientific knowledge it tries to provide a guideline for the policy on public health.

In addition to an internal secretariat of about 25 collaborators, the Council has an extensive network of more than 500 experts (university professors, collaborators of scientific institutions) at its disposal of which 300 have been appointed as Council Experts; the experts convene in multidisciplinary working groups to draw up advices.

As an official organ, the Superior Health Council believes it is essential to guarantee the neutrality and impartiality of the scientific advices it provides. To this end, it has worked out a structure, rules and procedures that allow to efficiently meet these needs at every step of the creation of the advices. Key moments in doing this are the preliminary analysis of the demand, the assignment of experts for the working groups, the putting into place of a system for managing possible conflicts of interest (based on the declaration of interest, investigations of possible conflicts of interest and a Deontological Commission and the eventual validation of the advices by the Committee (the final decision-making body). This coherent whole should allow for the delivery of advices that are based on the highest possible scientific expertise available within the highest degree of impartiality.

The advices of the working groups are submitted to the Committee. After being validated, they are sent to the applicant and to the minister of public health and the public advices are published on the website (www.hgr-css.be). In addition, a number of the advices are communicated to the press and to target groups among health care practitioners.

The SHC is also an active partner in the EuSANH network (*European Science Advisory Network for Health*) that is currently under construction and which is intended to elaborate advices on the European level.

If you wish to stay informed on the activities and publications of the SHC, you can send an e-mail to info.hgr-css@health.belgium.be .

Disclaimer

The FASFC's Scientific Committee and the Committee of the Superior Health Council (SHC) at all times reserve the right to modify the advice by mutual consent, should new information and data become available after the publication of this version.