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Advice from the director Office for Risk Assessment & Research

To the Minister of Health, Welfare and Sport and Minister for Agriculture

Date 15 October 2014

Advisory report on the risks associated with the consumption of mass-reared insects

At both national and international level, it is being suggested that insects might provide a possible alternative source of animal protein. In many parts of the world, certain insect species are a traditional source of food. Insects contain protein, vitamins, minerals and fatty acids. They could, therefore, partially replace animal protein in people's diets. Insects for human consumption are currently being produced in the Netherlands, albeit to a limited extent.
The Member States of the European Union are deciding on whether insects should be considered novel foods. If in future insects are to be considered novel foods, in accordance with EC Regulation No. 258/97, then, as stipulated by that regulation, those insects (and insect products) intended for human consumption must complete a European marketing approval procedure. The European Commission has asked the European Food Safety Authority (EFSA) to provide a scientific opinion on the risks associated with the consumption of insect proteins. This scientific opinion is expected in 2015, at the earliest.

<u>Question posed</u>
The Director of the Division Consumer & Safety Division of the Netherlands Food and Consumer Product Safety Authority has asked the Director of the Office for Risk Assessment & Research (BuRO) for details of the chemical, microbiological and parasitological risks of consuming heat-treated and non-heat-treated insects.

<u>Research and differentiation</u>
In the context of risk assessment, discussions were held with experts in the field of edible insects at Wageningen University, and with members of the Dutch Insect Breeders Association (VENIK) on the topic of rearing insects. The goal was to take stock of those insects that are currently being supplied for human consumption and of the production processes involved. This risk assessment is limited to those insect species that are currently being reared for human consumption in the Netherlands: the mealworm beetle (*Tenebrio molitor*), the lesser mealworm beetle

(*Alphitobius diaperinus*), and the European migratory locust (*Locusta migratoria*). The draft advisory report was evaluated by various independent experts, none of whom were involved in the study or in the preparation of the advisory report.

Results

- Larvae of the mealworm beetle and the lesser mealworm beetle, with empty guts, are rinsed in water, frozen and freeze-dried, then packed in 50 gram boxes. Adult locusts, with empty guts, are frozen and freeze-dried, then packed in 28 gram jars.
- Pathogenic microorganisms may be present in the production environment or in the insects' feed. Accordingly, the possibility cannot be excluded that these bacteria are also present in untreated insects that are intended for consumption.
- The results of small-scale studies have shown that the aerobic total viable count and the maximum permissible concentration of *Enterobacteriaceae* in fresh insects exceed the process hygiene criteria for the raw materials used in meat preparations. When mass-reared insects were subjected to a ten-minute heat treatment, it was found that the aerobic total viable count and the concentration of *Enterobacteriaceae* fell to values that were in compliance with these process hygiene criteria.
- The packaging information indicates that these products have a shelf life of 52 weeks. However, there are no known studies of whether the product is, in fact, safe throughout this 52-week period.
- Given the current production methods, these insects are unlikely to be exposed to toxic substances through their diet or through the surroundings in which they are reared.
- It is unclear exactly how much chitin (N-acetyl-D-glucosamine) insects reared in the Netherlands contain, and whether there are any health risks associated with an intake of more than 2.7 grams of chitin per day. Depending on the percentage of chitin involved (estimated at 6%), the intake of a daily portion of 45 grams of freeze dried insects is not a cause for concern in terms of public health.
- Few studies have yet been carried out into allergenicity resulting from the intake of whole insects or of insect protein. No studies have been carried out into the effects that preparing insects has on allergenicity. The possibility cannot be excluded that, after eating insects, sensitive individuals may experience sensitisation and allergic reactions (including severe allergic reactions).
- One potential occupational risk for the staff of insect rearing facilities is that they might develop hypersensitivity or allergic reactions as a result of exposure to insects (or to insect body parts).
- To the best of our knowledge, no toxicological studies involving whole insects or insect protein have been carried out in human subjects or in experimental animals.

Conclusions

1. To meet the proposed process hygiene criteria (as applicable to the raw materials used in meat preparations), insects must be heated before being frozen and freeze dried.
2. The chemical, microbiological and parasitological risks of consuming insects can be sufficiently well controlled through the use of adequate production methods.
3. The possibility cannot be excluded that, after eating whole insects or insect protein, sensitive individuals may experience sensitisation and allergic

reactions (including severe allergic reactions).

4. One potential occupational risk for the staff of insect rearing facilities is that they might develop hypersensitivity or allergic reactions as a result of exposure to insects (or to insect body parts).
5. If the expected intake of dried or freeze dried, whole insects exceeds 45 grams per day, the risk of chitin intake should be reassessed.

Advisory report

To the Minister of Health, Welfare and Sport and the Minister for Agriculture

The purpose of the novel foods approval procedure is to test the safety of these foods before they are introduced to the market. Regardless of the European Commission's decision on whether insects are or are not novel foods within the framework of EC Regulation No. 258/97, and in view of the fact that insects for human consumption are already on sale in the Netherlands, my recommendations are as follows.

- Consider insects as foods that are required to comply with the hygiene regulations (EC Nos. 852/2004 and 853/2004), with the General Food Law Regulation (EC No. 178/2002), and with all other food-related legislation.
- Instruct the sector to draw up a hygiene code incorporating process hygiene criteria that are specific to the human consumption of insects. This hygiene code should describe all critical points in the production process and describe how hazards can be demonstrably controlled.
- Ask the Netherlands Food and Consumer Product Safety Authority (NVWA) to check that the sector has drawn up a food safety plan and/or a hygiene code, and whether the insect rearing facilities are compliant with this.
- Given the potential risk that insects may have allergenic properties, it is important that further research be carried out into allergenicity resulting from the intake of whole insects or insect protein. Although this is a task for the market stakeholders, the Office for Risk Assessment & Research has outsourced a study to answer part of this question.
- Bring this advisory report to the attention of the Inspectorate of Social Affairs and Employment, in relation to the risks of contact allergy and inhalation allergy to which workers in insect rearing facilities are exposed.

To the Inspector-General of NVWA:

When determining and monitoring a food safety plan and/or a code of hygiene for insect rearing facilities, effective management of the following critical points is a minimum requirement.

- Any products introduced to the market must have been heated, to reduce the microbiological risks involved. When assessing safety, use the process hygiene criteria for the raw materials used in meat preparations.
- The following food safety criteria can be used for commercial insects (or commercial insect products):
  - *Salmonella*: absent in 10 grams;
  - *L. monocytogenes*: <100 CFU/g;
  - *B. cereus, C. perfringens, S. aureus* and *Campylobacter* spp.: criteria as stated in the Preparation and Processing of Foodstuffs (Commodities Act) Decree.
- The shelf life of 52 weeks must be demonstrated with a special focus on microbiological safety, particularly with regard to spore-forming pathogens that can grow in the freeze-dried product.

Yours sincerely,

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

Substantiation

Background

According to FAO, insects can potentially help to solve the increasing demand for animal protein. Insects for human consumption (entomophagy) are currently being produced in the Netherlands, albeit to a limited extent. EFSA has tackled this subject ("Increase of edible insects in European diet possible"), and the European Commission (EC) has launched a debate about whether insects should be considered novel foods. The EC has asked the EFSA to deliver a scientific opinion on the risks of insect consumption in 2015 (verbal communication during the 52nd EFSA Advisory Forum meeting in Oslo, 18-19 June 2014).
Producers are responsible for the safety of the products they introduce to the market. One of the conclusions of the first International Conference on Insects to Feed the World (14 - 17 May 2014 in Ede, the Netherlands) was that there was an urgent need to develop legislation on edible insects to give those in the industry clear guidance on the requirements they have to meet.

Question posed

The Director of the NVWA's Consumer & Safety Division has posed the following question to the Director of the Office for Risk Assessment & Research (BuRO): What are the chemical, microbiological and parasitological risks of consuming heat-treated and non-heat-treated insects?

Approach and differentiation

In the context of this risk assessment, discussions were held with experts in the field of entomophagy, at Wageningen University, and with members of the Dutch Insect Breeders Association (VENIK) on the topic of mass-reared insects. The goal was to take stock of those insects that are currently being supplied for human consumption, and of the production processes involved. Risk assessment is limited to those insect species that are currently being reared in the Netherlands for human consumption. Based on a literature review, the current knowledge of the potential risks of insect consumption is described. The aim was to draw up a complete summary of the potential risks.
Comments have been added to the substantiation of the risk assessment and of the advisory report by members of staff at Wageningen UR (University & Research Centre), Medicines Evaluation Board (MEB), National Institute for Public Health and the Environment (RIVM), and NVWA, as well as by BuRO staff.

Risk assessment

<u>Introduction</u>

In the Netherlands, the contribution made by protein to total daily energy intake varies from 12% to 16%. Around 60% of the protein is of animal origin, while 40% is obtained from vegetable foods. The main sources of protein are meat (and meat products), followed by dairy products (including cheese), with cereal products in third place. According to the latest Dutch Food Consumption Survey, in terms of daily consumption, adult women consume 76 grams of meat (the biggest eaters consume 189 grams) while men consume 119 grams (the biggest eaters consume 278 grams) (van Rossum et al., 2011). Eating less protein is one possible solution to achieving a more sustainable diet. Another is protein transition, which involves a partial or complete transition from meat-based proteins to alternative, more sustainable sources of protein. Several companies in the Netherlands are rearing insects that could be used to replace (or partially replace) the animal protein in people's current diets.

Entomophagy (eating insects) has a long history in many parts of the world, including Africa, Asia and Latin America (van Huis et al. 2013a). With an estimated four million species, insects are enormously diverse. Across the world, people consume around 1,900 different species of insects (van Huis 2013b); these are usually collected in the wild, processed (by boiling, baking, or roasting) then consumed. Every stage of insect development (eggs, larvae, pupae and adults) is used for consumption (DeFoliart 1999). The most commonly eaten types of insects are beetle larvae (31%), caterpillars (18%), ants, bees and wasps (14%), locusts (13%), bugs (10%) and others, such as termites and dragonflies (14%)[4]. Edible insects contain protein, vitamins, minerals and fatty acids, but differ in terms of chemical composition and nutritional value, depending on the species and food source involved (Bukkens 1997, Finke 2002, van Huis et al. 2013a, Tong et al. 2011, Xiaoming et al. 2010). Their protein composition is similar to that of meat.

Oonincx et al. (2010) showed that, per kilogram of product, insects emit much lower quantities of greenhouse gases, such as methane (CH_4) and nitrous oxide (N_2O), than conventional production animals, such as cattle and pigs. Insects are more efficient than mammals at converting food into edible product, so they have a much smaller ecological footprint than standard production animals. The weight of feed required to produce 1 kg of edible weight is 2.1 kg for crickets, 4.5 kg for chickens, 9.1 kg for pigs and 25 kg for cattle (van Huis 2013b).

Hazard identification and characterisation

Like vertebrates, insects can contain biological agents and substances that can pose a risk to health if they are consumed by humans. Any microbiological and chemical risks will largely originate in the insect's environment. The risks will be greater with wild-caught insects than with those that live in a standardised mass-rearing environment, where exposure to biological agents and substances can be more effectively controlled. Unlike vertebrates, insects are usually eaten whole. The gut is not removed but is eaten along with the rest, as in shrimp, for example. Some insects contain naturally occurring toxins that, when consumed, could pose a risk to public health. In addition, insects can be exposed to toxic substances, through their feed or the environment, which can contaminate the final product. Finally, as with other foods, consuming insects could lead to allergic reactions.

Insect species and rearing methods in the Netherlands

Table 1 summarises the species, the stage of development, the nature of the final product and the corresponding quantities of insects produced in the Netherlands in 2012 for human consumption. In the Netherlands, the production and sale of insects for human consumption is still limited.

Table 1. Insects for human consumption in the Netherlands

Latin name	Dutch name	Common English name	Stage of devel-opment	Quantities produced
Tenebrio molitor	Meeltor	Mealworm beetle	Larva	approx. 1500 kg/year
Alphitobius diaperinus	Piepschuimkever	Lesser mealworm beetle	Larva	approx. 1000 kg/year
Locusta migratoria	Europese treksprinkhaan	Locust	Adult	approx. 45 kg/year (25,000 animals each weighing 1.8 gram)

The mealworm beetle and the lesser mealworm beetle are among those insects that, unlike locusts, for example, undergo a complete metamorphosis. The eggs of mealworm beetles and lesser mealworm beetles hatch into larvae which do not yet resemble the adult forms. The final larval moult is followed by the pupal stage. The pupa eventually splits open and the adult beetle emerges. Mealworms and lesser mealworms are grown on a nutrient medium consisting of bran mixed with flour or ground chicken feed, supplemented with carrots, potatoes and water. The (lesser) mealworm beetles lay their eggs in the bran. There are separate production processes for the growing larvae (mealworms) and the adult animals (beetles). Once the eggs have been laid, it takes eight to ten weeks (at a temperature of 28-30°C and a relative humidity of 60%) before the larvae can be harvested. A sieve is used to separate the larvae from their nutrient medium. They are then placed in a cool environment (6-15°C) for several days, without feed, to ensure that their guts are empty. The larvae are then rinsed clean in lukewarm water and rapidly frozen to -18°C. After freezing, the larvae are freeze-dried and packed in 50 gram boxes. The label bears a track and trace code (barcode) and a statement that the product must be thoroughly heated prior to consumption. The product's indicated shelf life is 52 weeks.

Locusts develop via a process known as incomplete metamorphosis. Their eggs hatch into nymphs which look very much like smaller versions of the adult stage. During successive moults, the insect becomes ever larger, until it ultimately reaches the adult stage. Migratory locusts lay their eggs in a peat substrate. After about ten days, the juveniles emerge from the egg pods and are transferred to production trays. They are fed on bran and fresh, dry grass. Depending on the temperature (22-25°C), the locusts are harvested after a period of 26-28 days. They are then mature, and are placed in a food-free environment for two days to empty their guts. They are then subjected to a rapid freezing and freeze-drying process. The locusts are then packed in 28 gram jars labelled with a track and trace number and the shelf-life expiry date. The label also indicates that the legs and wings should be removed prior to use and that the product must be heated.

Microbiological risks

Moulds, parasites, viruses and yeasts
Nutrient media are a good substrate for saprophagic moulds and bacteria. Pathogenic moulds such as *Aspergillus, Penicillium, Mucor* and *Rhizopus* can colonise insect nutrient media (Inglis and Sikorowski 2009). Entomophagy can enable parasites that use insects as intermediate hosts to cause infections in humans. Anyone eating raw or insufficiently heated, wild-caught insects can acquire a parasitic infection (Hinz 2001). The sterile initial stages of insects reared in the Netherlands for consumption are unlikely to be infected by viral or parasitic pathogens from their feed or from the surroundings in which they are reared (Belluco et al. 2013). The insect nutrient media used in the Netherlands are relatively dry, so they are not expected to contain moulds or yeasts in concentrations that might pose a risk to public health.

Bacteria
The gut microbiota of insects is made up of bacteria from their feed or from the surroundings in which they are reared (Dillon and Charnley 2002). Gut bacteria play an important part in maintaining insects in an optimal physiological state and in protecting them against pathogens. The number of bacteria present in the guts of larvae and adult insects ranges from 10^8 to 10^{11} per mg of gut contents (Cazemier 1996). This microbiota consists mainly of Gram-negative rod-shaped bacteria and Gram-positive cocci. No bacteria, yeasts or moulds were present in the guts of locusts (*Zonocerus variegatus*) and mealworm beetles (*Tenebrio molitor*) reared on a sterilised rearing medium or on a rearing medium to which antibiotics had been added (Ademolu et al. 2007). After a time, in conventionally reared mealworms (non-sterilised feed, no antibiotics), the gut (midgut) was found to contain about 10^5 CFU per ml of gut contents (Genta et al. 2006). The following bacteria were isolated from the guts of mass-reared locusts: three types of cocci – *Staphylococcus, Micrococcus, Streptococcus* – as well as *Bacillus subtilis, Pseudomonas* spp., and *Enterobacteriaceae* (*Klebsiella* spp., *Escherichia coli* and *Proteus mirabilis*) (Dillon and Charnley 2002).

A small number of *Enterobacteriaceae* consists of bacteria that can (depending on the numbers and species involved) cause disease in humans. As a result, they may pose a health risk to consumers of insects. It has been shown that bacteria such as *Salmonella* Enteritidis, *Escherichia coli* and *Campylobacter* spp. can establish themselves and proliferate in the insect gut (Leffler et al. 2010, Wales et al. 2010, Zheng et al. 2011). In most cases, the pathogens listed above were only excreted during a limited period of time. Lesser mealworms that had been exposed to *Salmonella enterica* excreted the bacterium in their faeces for a maximum of twelve days (Crippen et al. 2012). *E. coli* was excreted for six days (McAllister et al. 1996), and *Campylobacter* spp. for up to three days (Strohter et al. 2005, Templeton et al. 2006). The relatively high temperatures at which insects are reared may allow any of these pathogens that are present in the production environment or in the insects' feed to multiply. Accordingly, the possibility cannot be excluded that these bacteria are also present in untreated insects that are intended for consumption.

Microbiological process hygiene criteria
The concentrations of viable aerobic bacteria, *Enterobacteriaceae*, yeasts and moulds are often used as a measure of process hygiene. These process hygiene criteria are used by the producer to determine whether the critical points in the production process are being adequately controlled. Process hygiene criteria for the raw materials used in meat preparations (Hygiene Code for Butchers 2010), for minced meat (EC No. 2073/2005), or for cooked crustacean and molluscan shellfish (EC No. 2073/2005) could be used for insects. In the following part of this advisory report, the results of studies in insects are compared to the process hygiene criteria for the raw materials used in meat preparations. The aerobic total viable count and the number of *Enterobacteriaceae* which is used as a criterion for the raw materials used in meat preparations (after heating) are applicable, provided that the insects in question are indeed heated prior to sale. Table 2 illustrates the microbiological process hygiene criteria specified for foods of animal origin.

Table 2. Microbiological process hygiene criteria for meat and meat products

Product	**Hygiene code/legislation**	**Microorganism**	**Process hygiene criterion (CFU/g)**
Raw materials used in meat preparations	Hygiene Code for Butchers (Hygiene Code for Butchers. The Royal Dutch Butcher's Association, 2010)	Aerobic total viable count[a]	$<10^4$
		Aerobic total viable count[b]	$<10^6$
		Enterobacteriaceae[a]	$<10^2$
		Enterobacteriaceae[b]	$<10^3$
Minced meat[c]	Regulation on Microbiological Criteria for Foodstuffs (EC No. 2073/2005)	Aerobic total viable count	m[d] = 5×10^5 M[d] = 5×10^6
		E. coli[e]	m[d] = 50 M[d] = 500
Meat preparations[f]	Regulation on Microbiological Criteria for Foodstuffs (EC No. 2073/2005)	*E. coli*[e]	m[d] = 500 M[d] = 5000
Shelled and shucked products of cooked crustacean and molluscan shellfish	Regulation on Microbiological Criteria for Foodstuffs (EC No. 2073/2005)	*E. coli*	m[d] = 1 M[d] = 10
		Coagulase-positive staphylococci	m[d] = 100 M[d] = 1000

[a] Process step: cool after heating.

[b] Process step: presentation/sale of prepared products after heating.

[c] Boned meat that has been minced into fragments and contains less than 1% salt.

[d] Five subsamples have to be taken. The results are deemed adequate if all samples ≤m. The results are acceptable if two of the five subsamples have values between m and M. The criterion applies to the end of the production process.

[e] *E.coli* is used here as an indicator of faecal contamination.

[f] Fresh meat, including meat that has been minced into fragments, to which foodstuffs, seasonings or additives have been added or which has undergone processes that are not sufficient to modify the internal muscle-fibre structure of the meat, thereby eliminating the characteristics of fresh meat.

In minced meat and meat preparations, the process hygiene criterion for *E.coli* is an indicator of faecal contamination. Coliform bacteria in general, and *E.coli* in particular, are especially common in mammals and birds. Only about 15% of the coliform bacteria found in insects are comparable to the coliforms found in the above-mentioned animals (Geldreich et al. 1964). Accordingly, these cannot be used as a process hygiene criterion in the production of insects. In crustacean and molluscan shellfish, *E.coli* is primarily an indicator of pathogenic agents such as norovirus, which need not be assumed to pose a risk during the production of insects.

In addition to microbiological process hygiene criteria, insects should be periodically tested for the presence of specific pathogens. To this end, there are microbiological food safety criteria for a range of foods. Table 3 summarises the food safety criteria that are relevant to insects.

Table 3. Relevant food safety criteria

Product	Hygiene code/ legislation	Microorganism	Food safety criterion
Minced meat and meat preparations made from poultry meat and poultry meat products, intended to be eaten cooked	Regulation on Microbiological Criteria for Foodstuffs (EC No. 2073/2005)	*Salmonella*	Absent in 25 grams[a]
Minced meat and meat preparations made from other species than poultry, intended to be eaten cooked	Regulation on Microbiological Criteria for Foodstuffs (EC No. 2073/2005)	*Salmonella*	Absent in 10 grams[a]
Cooked crustacean and molluscan shellfish	Regulation on Microbiological Criteria for Foodstuffs (EC No. 2073/2005)	*Salmonella*	Absent in 25 grams[a]
Ready-to-eat foods that can serve as nutrient media for *L. monocytogenes*	Regulation on Microbiological Criteria for Foodstuffs (EC No. 2073/2005)	*Listeria monocytogenes*	<100 CFU/g or ml[a]
		Bacillus cereus	$<10^5$ CFU/g or ml
		Clostridium perfringens	$<10^5$ CFU/g or ml
		Staphylococcus aureus	$<10^5$ CFU/g or ml
		Campylobacter	Absent in 25 grams

[a] Five subsamples have to be taken. The results are deemed adequate if the microorganism is absent in the specified quantity of product. The criterion applies to commercially available products for the duration of their shelf life.

[b] These criteria do not apply a) to unprocessed, raw foods and drinks; and b) to processed foods and drinks that have not undergone any germicidal treatment and which, in normal use, are only suitable for human consumption after they have been heated by the end user.

Unlike warm-blooded animals, mass-reared insects are not likely to contain substantial numbers of *Salmonella* and *L. monocytogenes*. If a pathogenic contamination does occur, then this is likely to be the result of an infection from the production environment, and the actual numbers of bacteria involved will be minimal. It seems logical that insects should be periodically tested against the food safety criterion for *Salmonella* in minced meat other than poultry meat (absent in 10 grams) and *L. monocytogenes* in ready-to-eat foods (<100 CFU/g), in accordance with the Regulation on Microbiological Criteria for Foodstuffs (EC No. 2073/2005).

The same goes for the criteria as stated in the Preparation and Processing of Foodstuffs (Commodities Act) Decree for *B. cereus, C. perfringens, S. aureus and Campylobacter* spp. Given its relatively long shelf life of 52 weeks, the number of pathogens that can grow in the freeze-dried product should also be determined on the 'best before' date.

In 2010, nVWA carried out a small-scale survey into the microbiological status of 55 insect products (locusts, lesser mealworms, mealworms and a mealworm snack) that had undergone no treatment apart from freeze-drying, and which were intended for human consumption and were retailed as such (nVWA, internal communication). The aerobic total viable count and the concentrations of *Enterobacteriaceae* and *Bacillus cereus* are shown in Tables 4, 5 and 6.

Table 4. Aerobic bacteria: number of samples and percentage of samples (%) at each concentration level (CFU/g)

Product	**Number**	**$<10^5$**	**10^5-10^6**	**10^6-10^7**	**$>10^7$**
Locust	17	30%	41%	18%	12%
Lesser mealworm	17	24%	6%	53%	18%
Mealworm	17	12%	-	47%	41%
Mealworm snack	3	100%	-	-	
Total	54	26%	15%	37%	22%

Table 5. *Enterobacteriaceae*: number of samples and percentage of samples (%) at each concentration level (CFU/g)

Product	**Number**	**$<10^3$**	**10^3-10^4**	**10^4-10^5**	**$>10^5$**
Locust	17	59%	18%	24%	-
Lesser mealworm	17	24%	6%	41%	29%
Mealworm	18	11%	17%	28%	44%
Mealworm snack	3	100%	-	-	-
Total	55	35%	13%	29%	23%

Table 6. *B. cereus*: number of samples and percentage of samples (%) at each concentration level (CFU/g)

Product	Number	$<10^2$	10^2-10^3	10^3-10^4
Locust	17	88%	12%	-
Lesser mealworm	17	88%	6%	6%
Mealworm	18	100%	-	-
Mealworm snack	3	100%	-	-
Total	55	93%	5%	2%

It was found that 59% of the insect products tested (Table 4) exceeded the process hygiene criterion for aerobic bacteria in raw materials used in meat preparations (10^6 CFU/g), while the concentration of *Enterobacteriaceae* (Table 5) in 65% of the samples exceeded the criterion for raw materials used in meat preparations (10^3 CFU/g). Given the small number of bacteria found, the mealworm snack had probably undergone a heat treatment. The following specific, pathogenic microorganisms were not found in this small-scale survey: *Clostridium perfringens*, *Salmonella* and *Vibrio*. In 93% of the samples, the concentrations of the spore-forming bacterium *Bacillus cereus* were less than 100 CFU/g (Table 6).

Klunder et al. (2012) studied the microbiological status of fresh and heat-treated larvae of the mealworm beetle (*Tenebrio molitor*) that had been reared in the Netherlands. The aerobic total viable counts and the concentrations of *Enterobacteriaceae* and spore-forming bacteria in fresh mealworms (Table 7) were similar to those found by the nVWA study in freeze-dried mealworms.

Table 7. Average microbiota of whole, fresh and heat-treated mealworms (Klunder et al. 2012)

Colony forming units (CFU/g)	Fresh	Cooked (10 min.)	Roasted (10 min.)
Total number of aerobic bacteria	5.0×10^7	<50	<50
Enterobacteriaceae	6.3×10^6	<10	160
Bacterial spores	130	<10	40

Submitting mealworms to heat treatment substantially reduced the numbers of aerobic bacteria, *Enterobacteriaceae* and bacterial spores. Boiling the mealworms for ten minutes was more effective in reducing the quantities of *Enterobacteriaceae* and spores than roasting (Klunder et al. 2012). The study carried out by Klunder and co-workers (2012) also shows that the fresh insects studied would not meet the proposed process hygiene criterion for aerobic total viable count of $<10^6$ CFU/g and for *Enterobacteriaceae* of $<10^3$ CFU/g. However, the use of heat treatment (boiling or roasting) ensured that the process hygiene criteria were met.

Storage, shelf life and preparation
Insects supplied for consumption in the Netherlands are not routinely heated

during the production process, they are just frozen and freeze-dried. The packaging information indicates that these products have a shelf life of 52 weeks. However, there are no known studies of whether the product is, in fact, safe throughout this 52-week period.

Chemical and nutritional risks

Adult mealworm beetles have special glands in their abdominal cavity that can secrete defensive substances (Brown et al. 1992, Ladisch et al. 1967). These defensive secretions contain benzoquinones, which have been described as toxic, carcinogenic and mutagenic to animals (Lis et al. 2011, Wirtz and Fruin 1982). In mice, quinones have been found to have an LD50 of 50-300 mg/kg body weight (ref. Wirtz and Fruin 1982). The International Agency for Research on Cancer (IARC) has classified 1,4-benzoquinone as a Group 3 substance, 'not classifiable as to its carcinogenicity to humans'. To the best of our knowledge it is only the adult beetles that contain quinones, so the larvae currently being used as food for humans and animals contain no quinones.

Several studies have investigated the chemical composition of insects. These focus primarily on nutrients. Grabowski et al. (2010) examined the chemical composition of three insect species that are sold in pet shops. In the case of one species of locust (*Schistocera gregaria*) and one species of cricket (*Acheta domesticus*), it was concluded that their chemical composition posed no obstacle to human consumption. This was not true of the third species in the study, *Phymateus saxosus*, which is known to be toxic.

An insect's diet determines its chemical composition (Oonincx et al. 2011) and insects can accumulate substances such as PCBs and dioxins (Devkota and Schmidt 2000, Hunter et al. 1987, Jamil and Hussain 1992). Besides nutrients, artificial nutrient media contain substances that are added to improve their stability, palatability and nutritional value. In addition, antimicrobial agents (fungicides and antibiotics) may be added to combat microbial contaminations (Inglis and Sikorowski 2009). There is considerable interest in the Netherlands in developing production lines that use residual organic waste as the insect growth medium. The chemical composition of such residual waste might pose a risk, as the presence of harmful metals, pesticides, etc. could result in the chemical contamination of mass-reared insects. The production method currently used in the Netherlands involves very little risk that insects intended for human consumption will be chemically contaminated through their diet or through the surroundings in which they are reared, especially if the ingredients for the feed and the nutrient medium are obtained from GMP-certified companies.

Consumption of the roasted larvae of the African silkworm (*Anaphe venata*) was suspected to be the cause of a seasonal ataxia in southwestern Nigeria (Adamolekun 1993). It has now been shown that these insects contain thiaminases, which break down thiamine (vitamin B1). An unbalanced diet, in combination with a thiamine deficiency, could have led to the ataxia (Nishimune et al. 2000). Thiaminases are highly resistant to heat, which underlines the importance of using an effective heat treatment to convert these insects into a safe food source.

Chitin
Chitin (N-acetyl-D-glucosamine) is found in the insect exoskeleton and has a protective function (EPA 2007). Dried insects contain about 10% chitin (Arbia et al. 2012, DeFoliart 1992). However, other publications indicate higher (Lease and Wolf 2010) and much lower chitin contents (Finke 2007), depending on the analytical method used. Indigestible chitinous body parts can accumulate in the bowel, causing partial or total constipation (van Huis et al. 2013a). For this reason, it is recommended that the legs and wings of locusts be removed prior to consumption.

In 2010, the EFSA Panel on Dietetic Products, Nutrition and Allergies issued a scientific opinion on the safety of chitin-glucan (consisting of D-glucosamine, N-acetyl-D-glucosamine and glucose) as a novel food ingredient. This ingredient, which is derived from the cell walls of *Aspergillus niger*, is intended to increase daily fibre intake. Given that the intended chitin-glucan intake amounted to 2 to 5 grams per day, there were no safety concerns with regard to public health (EFSA 2010). Chitin-glucan makes up more than 90% of the product, and the ratio of chitin to glucan is 60:40. Intakes in excess of 5 grams of chitin-glucan per day were not investigated. The toxicological data on which the EFSA's scientific opinion was based were generated in studies of chitin derived from crustaceans. To our knowledge, no such research has been carried out on chitin derived from insects. Glucosamine has low acute and chronic oral toxicity and there is no evidence to suggest that it is either genotoxic or allergenic (EFSA 2009). It is not possible to say, with any degree of certainty, just how much chitin the mass-reared insects examined in this advisory report contain, but there is evidence to suggest that it amounts to about 5-6% of the dry weight (Tumewu 2012). This means that the consumption of 45 grams of freeze-dried, whole insects per day is not a cause for concern in terms of public health.

Allergenicity
The possibility that novel proteins or other components of a novel food might give rise to allergic reactions should be carefully examined (97/618/EC). In this context, allergy is defined as a response to food by the human immune system, which is mediated by immunoglobulin E (IgE). The more familiar allergic reactions are those caused by insect bites or by stings from bees and wasps, for example. Some allergic reactions are also attributed to contact with insect body parts or insect waste (contact allergens) or others are the result of inhaling dust particles consisting of pulverised carcasses, shed skins, or faeces (inhaled allergens). The symptoms of such allergies range from eczema and dermatitis to asthma and anaphylactic shock. It is known that working at an insect rearing facility can induce hypersensitivity or an allergic reaction (Harris-Roberts et al. 2011). Bellas (1999) estimated that 30% of the staff at insect rearing facilities had health problems of this kind.

Human allergic or hypersensitive reactions after inhaling insect body parts have been reported for 150 species of insect (Bellas 1999). In Bulgaria, people working in the walnut industry developed eczema, dermatitis and severe itching as a result of exposure to the larvae and faeces of the Indian Meal Moth (*Plodia interpunctella*). A study involving USDA laboratories where insects are reared identified nine species of mites and spiders as the source of substances that, when inhaled, can generate allergic reactions. Despite wearing protective clothing and masks, more than half of the staff developed allergies to insects (or to insect body parts). Numerous cases of asthma have been reported among those who work with *Lepidoptera* (Reinecke 2009). Reactions have been reported to insects of the orders *Orthoptera* (which include cases of anaphylactic shock), *Coleoptera*, *Diptera*, *Ephemeroptera* and *Trichoptera*. Cockroach allergy, which is associated with the development of asthma as a result of inhaling cockroach allergen, has been well described (Pomes et al. 2007, Wirtz 1984). Hypersensitivity to crickets has been described (Siracusa 2003) and mealworms, too, have been identified as a source of allergens (Ledent and Mairesse 2006). The presence of IgE antibodies specific to a moth (*Bombyx mori*) and a midge (*Chironomus yoshimatsui*) was detected in asthma patients with a sensitivity to house dust and house dust mites. This work demonstrated that these two insects carry important specific allergens and that cross allergenicity occurs between the two species (Komase et al. 1997).

Accordingly, there is compelling evidence that, when inhaled, materials from a large number of insect species can generate an allergic reaction (Mathews 1989). This is an occupational hazard for workers who are exposed to insects. The preventive measures to be taken include ensuring that there is proper ventilation, and wearing protective clothing and masks. Sensitised individuals should suspend their normal working activities.

Little is known about allergic reactions resulting from eating or accidentally swallowing insects. Small amounts of insect material will be present in a range of foods, such as those based on cereals and fruits. This means that people are at risk of becoming sensitised. If they go on to eat larger quantities of insects, this may eventually provoke an allergic reaction, as the risk of sensitisation increases with exposure. Allergens can survive both high temperatures (e.g., cooking) and the levels of acidity found in the stomach. One study involving a small number of subjects (Freye et al. 1996) demonstrated that people were sensitised after eating the larvae of mealworm beetles (*Tenebrio molitor*) and darkling beetles (*Zophobas morio*).

Cross-reactivity can occur between two types of allergens. This means that IgE antibodies generated in response to one allergen can also bind to other allergens, thus inducing an allergic reaction. As a result, eating crustacean and molluscan shellfish such as shrimps, crabs, lobsters, squid, snails and oysters can cause an allergic reaction in susceptible individuals (MacEcvilly 2000). The most important allergen is the muscle protein tropomyosin. This protein has also been found in insects (cockroaches) (Reese et al. 1999) and other invertebrates, such as crustacean and molluscan shellfish, arachnids (house dust mites) and molluscs. Some individuals who were allergic to house dust mites became sensitive to tropomyosin from seafood. This observation suggests that people with seafood allergies may experience an allergic reaction after consuming edible insects.

The risk of allergic reactions to insect proteins was investigated in the SUPRO2 project conducted by Wageningen UR (University & Research Centre) and in a joint project by the Netherlands Organisation for Applied Scientific Research (TNO) and the University Medical Center Utrecht (UMCU). In addition to primary allergic reactions, tests are being conducted to determine whether cross-reactions can occur in those who are allergic to crustaceans or to house dust mites. Initial results show that antibodies from people with crustacean or dust mite allergies also react to, amongst others, tropomyosin from mealworms.

Conclusions

General

- This advisory report is limited to the following insects that are currently being reared for human consumption in the Netherlands: the mealworm beetle (*Tenebrio molitor*), the lesser mealworm beetle (*Alphitobius diaperinus*), and the European migratory locust (*Locusta migratoria*).
- Larvae of the mealworm beetle and the lesser mealworm beetle (which are also known as mealworms) are rinsed clean in lukewarm water, frozen and freeze-dried, then packed in 50 gram boxes. Adult locusts with empty guts are frozen and freeze-dried. The locusts are then packed in 28 gram jars.

Microbiological and parasitological risks

- There is no evidence to suggest that insects reared under hygienic conditions and on relatively dry nutrient media are contaminated with moulds or yeasts, or with viral or parasitic pathogens, and might therefore pose a risk to public health. Yet pathogenic microorganisms may well be present in the production environment or in the insects' feed and, if so, the relatively high temperatures used when rearing insects would allow them to multiply. Accordingly, the possibility cannot be excluded that these bacteria are also present in untreated insects that are intended for consumption.
- The same process hygiene criteria for raw materials used in meat preparations could be applied to insects.
- The results of small-scale studies have shown that the aerobic total viable count and the maximum permissible concentration of *Enterobacteriaceae* in fresh insects exceed these process hygiene criteria. Tests for two species of spore-forming bacteria, *Clostridium perfringens* and *Bacillus cereus*, showed that the former was not present in insects, while concentrations of the latter were in compliance with the food safety criterion.
- When mass-reared insects were subjected to a ten-minute heat treatment, it was found that the aerobic total viable count and the concentration of *Enterobacteriaceae* fell to values that were in compliance with the proposed process hygiene criteria.
- The packaging information indicates that these products have a shelf life of 52 weeks. However, there are no known studies of whether the product is, in fact, safe throughout this 52-week period.

Chemical and nutritional risks

- The adult form of the mealworm, the mealworm beetle, can contain toxic quinones. However, these substances are not present in mealworms, which are reared for human consumption. Given the current production methods, these insects are unlikely to be exposed to toxic substances through their diet or through the surroundings in which they are reared.
- It is unclear how much chitin (N-acetyl-D-glucosamine) insects reared in the Netherlands contain. Depending on the percentage of chitin involved (5-10%), the intake of a daily portion of approximately 45 grams of freeze dried insects involves no safety concerns with regard to public health. At higher intakes, the risk should be reassessed.
- Few studies have yet been carried out into allergenicity resulting from the

intake of whole insects or of insect protein. No studies have yet been carried out into the effects that preparing insects has on allergenicity. The possibility cannot be excluded that, after eating insects, sensitive individuals may experience sensitisation and allergic reactions (including severe allergic reactions).

- One potential occupational risk for the staff of insect rearing facilities is that they might develop hypersensitivity or allergic reactions as a result of exposure to insects (or to insect body parts).

<u>Toxicological risks</u>
To the best of our knowledge, no toxicological studies involving whole insects or insect protein have been carried out in experimental animals.

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