

The Director General

Maisons-Alfort, 12 February 2015

OPINION of the French Agency for Food, Environmental and Occupational Health & Safety

on "the use of insects as food and feed and the review of scientific knowledge on the health risks related to the consumption of insects"

ANSES undertakes independent and pluralistic scientific expert assessments.

ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.

It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are made public.

This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated on 12 February 2015 shall prevail.

On 23 June 2014, ANSES issued an internal request to conduct an expert appraisal on "the use of insects as food and feed and the review of scientific knowledge on the health risks related to the consumption of insects (2014-SA-0153)".

1. INTRODUCTION

ANSES's mission is to conduct health risk assessments in its area of activity and to provide the competent authorities with any information about these risks, as well as the expertise and scientific and technical support necessary to draft legislative and statutory provisions and implement risk management measures.

Several international organisations have recently commented on the value of insects as a source of human food and animal feed. In addition, the industry, especially in France, sees it as an opportunity and has started to offer products derived from insects. Following on from other European agencies, particularly those in Belgium and the Netherlands, which recently published their own reports on the safety of insects intended for human food, the French Health & Safety Agency decided to conduct a review of scientific knowledge and the benefits/risks associated with the consumption of insects.

A literature study conducted by the University of Liège-Gembloux was used to review existing scientific knowledge.

This Opinion addresses issues relating to health hazards (biological, physical, chemical, allergyrelated), etc.) in insects and products derived from insects intended for human food and animal feed. The nutritional and environmental aspects are discussed briefly.

The following points were not included in the expert appraisal:

- The question of the impact of insects on food security;
- The assessment of the health risks for individual insect species or products derived from insects;
- Consumption in the form of protein preparations (insect extracts);
- Issues relating to the welfare of insects at the different rearing and production stages;
- Problems related to biosafety in farms;
- Insect/plant interactions and plant health;
- Entomopathogens and insect health;
- The health risks associated with the collection of edible insects from the environment (wild capture).

2. GENERAL CONTEXT

2.1. The FAO has spoken out in favour of deriving value from insects

By 2030, over nine billion people will need to be fed, along with the billions of animals raised each year for food, recreation or as pets (FAO 2009). The United Nations Food and Agriculture Organization (FAO) has therefore highlighted a potential problem of global food security, which will probably be felt most acutely in developing countries (Belluco, Losasso *et al.* 2013).

Among the possible answers to the problems of sufficient food resources for humans and animals, in its report entitled "*Edible insects*", the FAO recommends considering rearing insects on an industrial scale (van Huis, van Itterbeeck *et al.* 2013). According to the FAO, nearly 2.5 billion humans regularly eat insects in the world. In many parts of the world, with urbanisation and the change in food habits, this number is in decline (Gracer 2010). In Europe, since this report was released, the idea of developing industrial production of edible insects has been gaining ground (van Huis, van Itterbeeck *et al.* 2013).

Several publications describe insects as ubiquitous and able to reproduce rapidly (i.e. *Acheta domesticus* can lay up to 1500 eggs in a month) throughout the year in controlled conditions. According to these same publications, moreover, insects exhibit high growth rates and feed conversion rates compared with conventional livestock farming (Nakagaki and Defoliart 1991). Nevertheless the methods of calculation that led to these results may need to be assessed. Other studies emphasise a low environmental impact throughout their entire life cycle, mainly because of lower greenhouse gas emissions (Oonincx, van Itterbeeck *et al.* 2010) and reduced demand in terms of rearing space. The publications conclude that some insects are highly nutritious foods, particularly rich in calories and with a high protein, fat and mineral content. If the insects were reared industrially, studies foresee organic waste, for example food waste (whose use is currently

prohibited by the regulations), being used as feed for insects. In addition, these insects can be consumed whole, ground into powder or paste form and incorporated in various food preparations to increase their acceptability to consumers (Yi, Lakemond *et al.* 2013).

2.2. A point of view supported by industry

In a context of increasing scarcity of resources, a decline in agricultural land and Europe's strong dependence on protein for animal feed, many animal feed manufacturers have shown a growing interest in the value of insects as a source of protein. They consider that the use of organic matter—waste and/or by-products of agriculture and the food industries—to rear insects could be worthwhile for several reasons: (1) exploitation of low-value products (manure, kitchen or agri-food industry waste) to produce a high-value source of protein, (2) reduction of large volumes of waste (for instance by vermicomposting, the transformation of biodegradable waste into natural fertilisers using compost worms). They foresee the rearing of several species of Diptera to help reduce various types of organic waste, in a short timeframe (van Huis 2013).

According to the industry, the use of insects as feed in aquaculture and poultry farming is expected to become more widespread in the next few decades. Traditional feed production for domestic animals should intensify further. They consider that, faced with the increasing scarcity of resources (land, water, etc.) and the need for a more efficient use of these resources, the time has come to diversify with, in particular, the use of new sources of protein (van Huis 2013).

2.3. Latest developments: research projects under way on this topic

In view of its current popularity among certain international institutions and manufacturers, French and EU research projects have been undertaken to improve scientific knowledge on this topic.

2.3.1. <u>ANR – DESIRABLE Project:</u> Design of an insect biorefinery to contribute to more sustainable agri-food systems.

This project aims to develop a refinery for the bioconversion by insects of undervalued protein byproducts suitable for animal feed. It was launched in 2013 and will continue for 48 months. Several public (five INRA laboratories, CNRS, CEA, ITAP) and private partners (Ynsect, IPV Foods) are involved in the project. ANSES, alongside other public institutions, expertise clusters, consumer organisations and representatives of the production sectors, is a member of the advisory committee of this project, which is being funded by the ANR.

Two insect species, the mealworm, *Tenebrio molitor* and the black soldier fly, *Hermetia illucens*, were selected. The research themes focus on the challenges of a sustainable food, especially the health and nutritional aspects.

2.3.2. <u>PROteINSECT – FERA (Food and Environment Research Agency)¹ Project</u>: "Insects as sustainable sources of protein" or how the exploitation of insects could provide a sustainable alternative supply of protein for animal feed and human nutrition.

This three-year project funded by the European Commission is based on an international consortium with public and private partners in Europe, Africa and Asia.

Two species of fly, the black soldier fly, *Hermetia illucens*, and the housefly, *Musca domestica*, are being studied and their larvae used in production to recycle organic waste into fertiliser. Other

¹ <u>http://www.proteinsect.eu/</u>

compounds can be extracted from the biomass of insects apart from protein intended for animal feed: chitin for its antimicrobial action and lipids for the production of biodiesel. Among the study themes, issues relating to the following were addressed:

- Industrial production of insects, processing and testing of animal feed;
- Assessment of the quality and safety aspects (environmental contaminants such as heavy metals, dioxins, PCBs and PAHs; pathogens and spoilage agents; chemical residues such as pesticides and veterinary drugs; allergens);
- The life cycle analysis (mainly the environmental aspects, sometimes economic and societal aspects).

The study is under way and the results are expected in the first quarter of 2016.

2.4. Regulatory context

The exploitation of insects is covered by several regulatory texts, including those on livestock, animal by-products, animal feed and novel foods for humans. The various regulations are presented in detail in Annex 1 of this Opinion and are summarised in this section.

Concerning the rearing of insects, there are no regulations specific to this type of farming. As these insects are non-domestic species, they fall within "captive wildlife" regulations². Operating a professional insect breeding facility therefore requires prior granting of a farming competency certificate and a prefectural authorisation to open. It should be noted that there are no national animal protection measures specific to insects reared in captivity.

Furthermore, they must not be reared on prohibited substrates (Regulation (EC) No 767/2009 (2009); Regulation (EC) No 1069/2009 (2009)).

Facilities and plants likely to generate risks or hazards are subject to specific legislation and regulations, relating to what are called "classified installations for the protection of the environment" (ICPE). Their activities are listed in a nomenclature that subjects them to a regime of authorisation³. More specifically, Section 2150 of this nomenclature relates to worm farms and insect or insect larvae farms whose purpose is to provide bait for fishermen, or food for pet birds, reptiles, etc.

Concerning the use in animal feed of invertebrates (and therefore insects) that are non-pathogenic to humans, they are classified as Category 3 material as defined in Article 10.I of Regulation (EC) No 1069/2009 (2009) but are not regarded as processed animal proteins (PAPs). Restrictions on use are thus statutorily provided for by Regulation (EC) No 999/2001 (2001) which prohibits the use of PAPs in livestock feed, with the exception, since 1 June 2013, of feed for aquaculture animals (Regulation (EC) No 56/2013 (2013)). It should be noted that this regulation does not apply to petfood nor feed for fur animals. If the legislation were to evolve to include insects in PAPs, their use would be permitted for fish or even, depending on regulatory developments, non-ruminant animals (pigs, poultry).

² French Environment Code, <u>Articles L. 413-1 to L. 413-5 (PDF - 59 kb)</u> and <u>Articles R. 413-1 to R. 413-50 (PDF - 92 kb)</u> and its implementing texts.

³ <u>http://www.installationsclassees.developpement-durable.gouv.fr/accueil.php</u>

The regulatory status of insects as food for humans raises some questions. The use of insects as human food comes under Regulation (EC) No 258/97 (1997) of the European Commission concerning novel foods and novel food ingredients⁴ and must therefore be subject to the authorisations required by this text. The current regulation is imprecise, since it covers only parts of animals (and not whole insects), and is ambiguous (difficulty interpreting the "significant degree" of consumption prior to 1997, which is the basis of this regulation). A revision of the regulation is scheduled for 2016. Whole insects and parts of insects should be mentioned in it very clearly. To this day, no application for authorisation has been validated at European level. Therefore, no insect or insect derivative can be placed on the market for human food in strict compliance with the regulations currently in force.

However, faced with the rising interest aroused by the exploitation of insects, some manufacturers in Europe have exploited the ambiguity of the current texts to exempt themselves from the procedures prior to their marketing. Thus, for example, the Belgian Federal Agency for the Safety of the Food Chain (FASFC) has already authorised the marketing of ten insect species and their derivatives for human consumption in Belgium. Under the guise of compliance with the general food legislation rules in force, since December 2013 Belgian companies have benefited from a "tolerance", pending European harmonisation for marketing authorisation in the EU market as a whole.

3. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

A research and development agreement (CRD) was signed between ANSES and the University of Liège-Gembloux to enable the Functional and Evolutive Entomology Unit of Gembloux Agro-Bio Tech to conduct a literature review of scientific and regulatory knowledge on the consumption of insects and their derivatives.

The intermediate document submitted in January 2014 was reviewed by a group of experts from the Expert Committees (CES) respectively on biological risks (BIORISK), chemical risks (ERCA) and animal feed (ALAN). The final document was presented in a plenary meeting to the CES on BIORISK on 20 May 2014 and to the CES on ALAN on 17 June 2014. The final document, taking into account the requested modifications, was submitted by Gembloux Agro-Bio Tech on 7 July 2014.

For the purposes of this Opinion, the collective expert appraisal was carried out by the CES on BIORISK and the CES on ALAN on the basis of an initial report, prepared by the group of experts that reviewed the document produced under the CRD and presented at plenary sessions from September to December 2014. The final report was validated on 10 December 2014.

ANSES analyses the links of interest declared by the experts prior to their appointment and throughout the work, in order to avoid potential conflicts of interest with regard to the matters dealt with as part of the expert appraisal.

The experts' declarations of interests are made public via the ANSES website (www.anses.fr).

⁴ "Novel food" is food which was not consumed in the EU to a significant degree before May 1997.

4. ANALYSIS AND CONCLUSIONS OF THE CES

4.1. General points

4.1.1. Human consumption of insects in the world

a. Intentional consumption

Currently, 2086 species of insects are consumed by around 3071 ethnic groups in 130 countries of the world (Ramos-Elorduy 2009; Rumpold and Schlüter 2013). In countries in tropical regions, in Africa, Asia, Australia and South America, "entomophagy"⁵ traditionally developed in rural populations, which found a very cheap and abundant source of protein in insects harvested from the wild (Barre, Caze-Subra *et al.* 2014). It then arrived in the frequently overcrowded cities of these various countries, often among the poorest urban populations. While the collection of edible insects still persists in rural areas, it has given way to an industry of mass production of edible insects, mainly located in peri-urban areas. Developed initially in modestly sized farms, this production and rearing of edible insects now takes place in specialised companies, especially in Thailand and other countries in Asia (Barre, Caze-Subra *et al.* 2014).

The most commonly consumed insects are:

- Larvae or adults of Orthoptera (crickets, locusts and katydids) and Hymenoptera (bees, wasps and ants),
- Larvae of Coleoptera (weevils and longhorn beetles),
- Caterpillars and pupae of Lepidoptera (butterflies and moths),
- And also some adults of Isoptera (termites) or aquatic Hemiptera (waterbugs) (Durst and Shono 2010; Mignon 2002; Raubenheimer and Rothman 2013).

There are no accurate data on the consumption of insects in France, which is very probably marginal. Although the aim of the various players in the sector is to incorporate insects in the basic diet in the future, it is still currently a niche market for a few consumers mainly keen to experience new sensations.

b. Unintentional consumption

The Codex Alimentarius standards relating to cereals, dried vegetables, legumes and plant protein materials prohibit the presence of whole live insects in flour or grains, but authorise a maximum of 0.1% of insect fragments by mass of the sample. Also taking this reality into account, the United States Food and Drug Administration (FDA) has defined tolerances for these types of faults, which are natural and inevitable in some foods⁶.

In this regard, the entomologist Marcel Dicke of the University of Wageningen in the Netherlands has estimated our involuntary annual consumption of insect fragments to be between 500g and one kilogram, especially in manufactured products containing flour (bread, pasta, biscuits, etc.), chocolate, fruit and fruit juice, or vegetables.

⁵ "Entomophagy": from the ancient Greek *entomos*: insect and *phagos*: eat; refers to consumption of insects by the human species. Comby B (1990) 'Délicieux insectes. Les protéines du futur.' (Paris)

http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/SanitationTransportation/ucm0 56174.htm

4.1.2. Industrial applications

Some insects regarded as crop pests are nevertheless valued throughout the world (Defoliart 1995). For example, sericulture exploits the silkworm, the caterpillar of the mulberry silk moth (*Bombyx mori*) which feeds on mulberry leaves until the stage of production of the silk used to spin its cocoon. The silkworm has undergone intense domestication due to its technological and food potential. Several countries including China have invested in its production for obtaining silk and exploiting the pupae for human food and animal feed (Defoliart 1995). Since 1987, the Thai Ministry of Public Health has authorised the incorporation of *Bombyx mori* pupae in food formulae prescribed to malnourished children (Defoliart 1995). In India, Japan, Sri Lanka and China, the pupae of *B. mori* as well as residues from their production are used for feeding fish and poultry (Kiuchi and Tamaki 1990).

Another insect which has been successfully domesticated is the honeybee, *Apis mellifera*. In industrialised countries, beekeeping is practised for the production of honey but also of beeswax, pollen, propolis, royal jelly and bee venom (used to treat severe allergies related to stings) (Schmidt and Buchmann 1992). In tropical countries, in addition to honey, populations consume bee brood (larvae and pupae).

The colouring E120 is widely used in the agri-food and cosmetics industries. It comes from the cochineal *(Dactylopius coccus)*. This insect produces carminic acid to protect it from insect predators. Carminic acid is extracted from the body and eggs of this insect to make a red-coloured dye, crimson, authorised as a colouring by European regulations and used in various food formulations such as yoghurt, confectionery and sodas (Cardon 2003; Verkerk, Tramper *et al.* 2007).

Certain criteria have been selected by professionals for the profitable production of edible insects: the choice would depend on a species with nutritional interest, while favouring characteristics that are more readily acceptable by human consumers (Rumpold and Schlüter 2013b). They may also be selected on the basis of their size, social behaviour (reduced cannibalism), safety for handlers, sensitivity to epidemic risks, potential for reproduction and survival, nutritional benefits, potential for storage and marketability (Schabel 2010). Generally speaking, they expect the target insect to produce many eggs with high viability rates, to develop quickly with maximum synchronisation of pupation in order to facilitate the harvesting of individuals, to have a high feed conversion rate, with inexpensive feed, to not be susceptible to disease, to be able to live in reduced spaces with a high density of individuals, and to produce quality protein compared to other animal or vegetable proteins (Rumpold and Schlüter 2013b).

The caterpillars of Lepidoptera are often used because they have no wings at this stage and do not jump, and they efficiently convert plant biomass into animal biomass (Schabel 2010). Orthoptera (locusts, katydids and crickets) are also used because of their abundance and geographical availability (Defoliart 1995). For example, one kilogram of biomass from the grasshopper *Oxya fuscovittata* was produced in 29 to 35 days from 84 individuals (Haldar, Das *et al.* 1999).

4.1.3. Taking nutritional aspects into account

The few publications on the subject highlight the nutritional value of edible insects, although these studies relate to only a very limited number of species. These results should therefore be regarded with the necessary precaution. It seems that some species are particularly high in calories, rich in protein, lipids, minerals and vitamins, and with amino acid compositions that are generally well

balanced for human needs (Raubenheimer and Rothman 2013; Rumpold and Schluter 2013a.) In contrast, insects are described as low in carbohydrates with a maximum of 10% of the total mass in some insect species (Chen, Feng *et al.* 2009). The nutritional composition and energy content of the main insect species consumed in various countries are presented in two recent publications (Makkar, Tran *et al.* 2014; Rumpold and Schluter 2013a.) The values provided are based on dry matter. The insects studied were analysed whole and always with their chitinous exoskeleton.

A study comparing 100g of insects with 100g of meat (fresh weight) reported a similar energy content (with the exception of some cuts of pork meat that are particularly rich in fat) (Sirimungkararat, Saksirirat *et al.* 2008).

The protein content may be equivalent to or even higher than the protein content of certain meats (Bukkens 1997; Ramos-Elorduy 1997; Srivastava, Babu *et al.* 2009). According to Rumpold and Schluter (2013a), proteins represent the main component of the dry matter of insects, between 45 and 75g/100g of dry weight, depending on the species. According to the same authors, the protein composition and amino acid content of insects vary greatly from one species to another. It also appears that insects are poor in methionine, which is rarely the case with meat. Testing of animal feed should make it possible to specify the quality of insect protein, both in terms of its digestibility and the efficiency of conversion into amino acids.

A Chinese study shows very wide variations in the lipid content of insects, between 7 and 77g/100g of dry weight, depending on the species considered and their diet (Chen, Feng *et al.* 2009). Lipid content may be higher in larvae and pupae than in adult insects (Chen, Feng *et al.* 2009). The insects with the highest lipid content usually belong to the orders of Isoptera (termites) and Lepidoptera (butterflies and moths). Compared to poultry and fish, insects may contain more polyunsaturated fatty acids (PUFAs), (DeFoliart 1991). In comparison, beef and pork contain very few PUFAs but far more monounsaturated fatty acids (MUFAs) (DeFoliart 1991).

The cholesterol composition also varies with the diet of the insects (Ritter 2010). Indeed, they are unable to produce their own sterols, which requires them to obtain them from their food.

The results obtained from a German study (Rumpold and Schluter 2013a) indicate that, in general, the daily calcium and potassium requirements for humans would not be met by the consumption of 100g of edible insects. As to the low amounts of sodium, it is conceivable that certain insects could be used in low-salt diets. Finally, the needs in copper, iron, magnesium, manganese, selenium, zinc and phosphorus could be met with the consumption of 100g per day of certain insect species. The presence of iron and zinc in insects is particularly interesting because these two minerals are often the cause of nutritional deficiencies in developing countries (Rumpold and Schlüter 2013b; van Huis, van Itterbeeck *et al.* 2013). The mineral content also varies, depending on the insect species, its stage of development and its diet (Rumpold and Schlüter 2013a; Rumpold and Schlüter 2013b; van Huis, van Itterbeeck *et al.* 2013). There are few data on vitamin composition but these seem to demonstrate very high variability. Nevertheless, some very carefully selected insects seem to be able to provide the vitamins needed by humans. In addition, rearing these insects on substrates that are rich in vitamins may increase the insects' vitamin content (Pennino, Dierenfeld *et al.* 1991).

As indicated above, the nutrient compositions of edible insects are generally subject to wide variations. External factors such as climate, food, habitat and preparation (for example whether insects are grilled or boiled), as well as the analytical method (Bukkens 1997; Chen, Feng *et al.* 2009; Verkerk, Tramper *et al.* 2007) also need to be taken into account. In order to mitigate these variations, if insects were to be incorporated in the human diet (especially that of people with

certain metabolic disorders), standards covering quantification, rearing practices and even diet composition should be developed and made available to farmers (Bednářová, Borkovcová *et al.* 2014).

4.1.4. Taking environmental issues into account

There are very few studies on the environmental impact of insect rearing. The development of a sustainable new insect sector must take the environmental footprint into account in the same way as the economic and societal aspects. This is one of the objectives of the ANR's "DESIRABLE" project currently under way, which is studying two species, *Hermetia illucens* and *Tenebrio molitor*.

The work by Oonincx and de Boer (2012) sought to quantify the ecological footprint of a Tenebrio molitor farm based in the Netherlands, through a life cycle assessment (LCA). The aim of a LCA is to assess the environmental impact of a system, by analysing all the processes related to the production cycle (manufacture and transport of food, fertiliser, energy, etc.). It is governed by the ISO 14040 and ISO 14044 Standards. Three parameters specific to operations within the facility were analysed and compared with conventional systems of livestock rearing (de Vries and de Boer 2010) and soybean production for animal feed (Dalgaard, Schmidt et al. 2007): the "global warming potential" (GWP), "fossil energy use" (EU) and "land use" (LU). According to the authors, for two of the three parameters studied (GWP and LU), the production of Tenebrio molitor appears to be less environmentally friendly than soybean cultivation but better than conventional animal production. It should be noted that this analysis does not take into account the qualitative nature of the land, which is exploited for animals such as cattle but would not be of much use for other types of production. The EU calculated would be almost identical for cattle and T. molitor, whereas pigs and poultry would require lower EU (soy was not classified due to a lack of data). This could be explained by the fact that the insects are poikilothermic (i.e. cold-blooded: they are unable to selfregulate their body temperature). Their development therefore depends on the rearing conditions and their thermal comfort zone. In the case of T. molitor, the optimal conditions are found at around 28°C and 70% relative humidity (Li, Zhao et al. 2013), which implies high energy consumption for a farm in western Europe for example. In addition, the insects have a high feed conversion rate (FCR) compared to warm-blooded animals on conventional livestock farms (van Huis 2010). Therefore, the farm's efficiency, in terms of weight gain at the selected stage, duration of growth, feed consumption and environmental impact, will require rigorous control of the temperature at the facility. This could lead to higher energy consumption than on conventional farms.

The environmental impact in terms of greenhouse gas production should also be taken into account. Around nine percent of CO_2 , between 35-40% of CH_4 , 65% of N_2O and 64% of NH_3 from total emissions produced by human activities, may be derived from conventional animal production (Steinfeld 2006). At the global level, production of bovine meat and milk may be responsible for the majority of total emissions produced by farms, respectively 41% and 20%. Meanwhile, emissions from pig or poultry production (meat and eggs) may respectively account for 9% and 8% of total emissions produced by farms (Gerber, Steinfeld *et al.* 2013). To date, only one study has compared production of greenhouse gases and ammonia between conventional cattle and pig farms and five different species of insects (Oonincx, van Itterbeeck *et al.* 2010). The results of the study indicate that the insect farms generally produce less greenhouse gas. Among the insects, it seems that only cockroaches, termites and scarab beetles produce methane gas (Hackstein and Stumm 1994). The others lack methanogenic bacteria in their digestive tract. Suitable selection of species could therefore reduce these emissions. According to De Vries and de Boer (2010),

production of CO_2 and N_2O is related primarily to the manufacturing process and transport of feed. Assuming that the insects have lower food needs, the quantities of gases they produce may be lower than those produced by pig and cattle farms. This study has two major limitations, however, since the analyses were performed over three days and not an entire lifetime, and it only included certain larval and nymphal stages of insect development.

Water is another natural resource to take into consideration when measuring the environmental impact of a farming practice. According to the FAO, agriculture already consumes up to 70% of the water extracted from groundwater, rivers and lakes (FAO 2011), and this fraction is expected to increase by 14% between 2000-2030 to meet growing food demand (FAO 2004). According to some authors, most insect farms require virtually no additional input in water, other than that contained in the feed (Feedipedia 2014; Siemianowska, Kosewska *et al.* 2013; Steinfeld 2006).

4.2. Rearing of edible insects

4.2.1. Collection

In many Asian, African and South American countries, gathering of wild insects (termites, ants, larvae, caterpillars, locusts) can meet local needs. This is a seasonal collection that is immediately followed by the consumption of the insects harvested. It enables many populations to enrich diets that are almost exclusively based on cereals (sorghum, millet or fonio in Africa) with low nutritional value in protein and lipids. Most of the time, these insects are eaten raw or grilled quickly. Naturally, this type of entomophagy, by gathering insects from the wild, is not subject to any health controls.

4.2.2. <u>Systems of rearing edible insects</u>

a. Introduction

Industrial farms of edible insects have mainly developed in Asian countries, especially Thailand and China. The FAO has provided information on farming techniques for various insect species (<u>http://www.fao.org/forestry/edibleinsects/fr/</u>). These industrial farms are often collection centres that retrieve production from small farms and process it industrially. The production of edible insects constitutes a recognised industrial activity, which feeds an internal market with strong demand (human consumption mainly), and is also increasingly looking toward exports. These companies mainly market insects in bulk or packaged in bags, and derived products (insect meal, insect-based confectionery, sugared insects, etc.). These different products are available from wholesaler distributors based in Europe, particularly in France.

The rearing of edible insects is gradually becoming established in Europe, especially in the Netherlands where, because of the strong relationships it has maintained with Asian countries, several companies specialised in the rearing of edible insects have emerged. Several industrial sites intended for the production of insects for human food or animal feed have developed in France. A French federation of insect producers, importers and distributors (FFPIDI) was set up in 2011 with the goal of structuring the insect sector.

b. Characteristics of the rearing of edible insects

The farming systems include:

- A strict containment system: insects intended for food are raised in tanks or trays, built from simple materials. They must prevent the passage of insects of any size, for example by means of muslin gauze or insect screens. Vivariums are used on a domestic or artisanal scale in Europe. Many species are cannibalistic and devour their own eggs and larvae if there is a shortage of food. Similarly, worms can eat nymphs. The separate rearing of adults and larvae, worms and nymphs is therefore strongly advised, with the organisation of specific nursery zones for egg laying and rearing of juveniles. For example, crickets are reared for a period of two months, and only the adults are collected and consumed. Conversely, mealworms are collected at the "end state" larval stage (approximately one month after laying), and adult Coleoptera are only used for reproduction.
- Maintenance of strict rearing conditions: constant ventilation, natural or artificial lighting is advisable with alternating day/night (some species feed mainly at night). Lamps for lighting also help maintain a sufficient temperature. Indeed, the temperature must be kept constant and rather high (a major determinant of growth in these cold-blooded animals): 25 to 30°C for example for crickets, in a temperate atmosphere. The relative humidity must be controlled to avoid encouraging the development of dust mites and moulds which increase mortality.
- A dry and rigid growing substrate for insects: untreated sawdust or wood chips, and/or structures in cellulose pulp, paper/cardboard derivatives, terracotta, etc. These substrates must contain cavities forming shelters (structures made from stacked terracotta tiles, from honeycombed cellulose pulp substrate, such as "preformed trays for packaging eggs" or from corrugated cardboard).
- *A source of drinking water.* either open water, provided in saucers, or moistened blotting paper or sponges soaked in water, which are easier to manage and essential for some species.
- A food supply adapted to the species' dietary preferences and processing capacity: food of plant origin, which most often means different plants (vegetables), plant products (meal) or waste from plants intended for human food (peelings) in dry form, supplemented by a fraction of very finely chopped fresh fruit or vegetables. Flaked cereals such as oat flakes for example, are particularly easy to use and are a suitable staple food. Crushed dry bread can also easily be used. In principle, a great many foods can be used as feed for insects, yet there are problems of appetite and acceptability of foods among insects and their larvae that are still not well understood (outside the entomology laboratories that rear insects and are familiar with these problems).

Conversion rates are apparently very favourable, but should be studied according to the species in order to choose the most suitable species. This conversion rate is regarded by companies as a critical indicator for considering large-scale, economically profitable production of insect protein.

It should be noted that mass production of live animals is generally accompanied by various zootechnical requirements, especially regarding health. In addition, the existence of entomopathogenic micro-organisms has been proven. Therefore, veterinary treatments intended to

improve the general health status of the animals should be considered. If such treatments are applied, it will be necessary to assess the risk presented by veterinary drug residues in the same way as for other animal species.

4.2.3. <u>Slaughter of edible insects</u>

In controlled farms, fasting of individuals from a few hours to a few days can sometimes precede slaughter, in order to ensure that the contents of the insects' digestive tract has been purged. However the effects on the intestinal microflora, depending on the species, their environments and their diets, have not been assessed.

Edible insects collected at the harvest stage (larvae, juveniles or adults depending on the species) are usually killed by one of two techniques:

- Freezing for at least 24 h at -18°C: this technique has little denaturing effect on proteins and better preserves the nutritional composition, but microbiological decontamination is not fully ensured, and parasitic decontamination (especially the presence of nematodes) is not always complete.
- Boiling: immersion in boiling water for 1 to 5 minutes, which cooks the insects while also ensuring their decontamination with highly efficient pasteurisation, is sufficient to destroy vegetative flora and parasites, but not bacterial spores. Some of the nutrients of interest may be degraded. The boiled insects are then drained and dried. The cooked insects do not keep well at this stage and should be processed immediately after boiling, or cooled immediately to 4°C for short-term intermediate storage. No data have been found on refrigerated storage of cooked insects. The cooked insects can however be frozen pending processing.

It should be emphasised that the development of such insect production sectors, from rearing through to slaughter, also poses the question of animal welfare. This topic has been explored very little in most invertebrates up to now.

4.2.4. Processing and preservation techniques for use in human food and animal feed

It is necessary to ensure that the processing techniques used on the insects do ensure their decontamination. Most edible insects offered for human food or animal feed are dehydrated after slaughter to preserve them. Several treatments may be used:

- Dehydration in a ventilated drying oven, at low temperature: the temperatures mentioned (Rumpold and Schluter 2013a) range from 60°C to 110°C. Dehydration at 90°C in dry air is often practised, for durations of more than five hours. At this temperature, a pasteurisation heat treatment is applied, with very high pasteurisation values. This obtains a satisfactory level of pasteurisation, even from thawed raw insects. In the latter case, the dehydration treatment also ensures cooking for insects that were not previously cooked. Frozen raw insects should preferably be thawed to 4°C before drying, with elimination of exudates, to avoid introducing any very low temperature products in the dehydration ovens.
- Deep-frying (more rarely used): this high-temperature (> 160°) heat treatment in boiling oil achieves more extensive microbiological decontamination of the products. The high

temperature also denatures protein-based venoms. It is practised on an artisanal scale in Asia, particularly for preparing Arachnids (spiders, scorpions), which are directly immersed in the frying fat. Some other insects such as locusts are prepared in this manner, especially in some areas of Africa. During deep-frying, the diversity of reactions (vitamin and pigment thermodegradations, Maillard reactions, etc.) and transformations generate new compounds of varying toxicity, including newly-formed compounds whose associated health risks should be assessed.

Toasting: higher drying temperatures are sometimes used at the end of the cycle (> 120°C) in order to "toast" the insects, develop specific aromas or improve the texture (intentionally crispy products for snacks). Treatments above 100°C, but in the absence of moisture, only destroy bacterial spores very partially: the products thus treated are only pasteurised, and viable spore contamination may still be present.

It should be noted that insufficient drying can lead to poor medium-term preservation, with the development of mould. In practice, the drying process should bring the insects to an $a_w < 0.7$ for adequate preservation, without development of mould during storage and with inhibition of any bacteria present.

Other methods may be considered, such as freeze-drying or acidification (Klunder, Wolkers-Rooijackers *et al.* 2012; van Huis, van Itterbeeck *et al.* 2013).

- Freeze-drying: this operation is used to eliminate by sublimation most of the water contained in a frozen product. It is therefore a low-temperature dehydration operation which enables foods to be preserved over the long-term (in a cool, dry place). Freeze-drying, because it is not regarded as a purifying practice, is unsuitable for insects killed by simple freezing, but could be considered for previously boiled insects.
- Acidification: Klunder, Wolkers-Rooijackers et al. (2012) showed that lactic fermentation could inactivate Enterobacteriaceae and stabilise the population of spore-forming bacteria in a mixture of composite flours and water containing between 10 and 20% of grilled ground mealworms (*Tenebrio molitor*) (a mixture for protein fortification of fermented foodstuffs). There are also accounts of vinegar having been used successfully to preserve foods derived from insects.

After dehydration, the edible insects can be kept at room temperature like any other dried product. After preservation they must be wrapped in a sealed package. The main factor limiting their preservation is the high amounts of unsaturated fatty acids that generate greater susceptibility to peroxidation due to oxygen (rancidity) under the effect of heat and light. The structure and presentation (divided solid, small particles of varying porosity) promote this oxidation, accelerating the development of rancidity in the products (unpleasant flavours, loss of nutritional quality). Products derived from insects are packaged in a neutral protective atmosphere (nitrogen) or vacuum packed to control their lifetimes. Some dehydrated products marketed in the European Union are vacuum packed and then frozen to slow the oxidation reactions.

Dried insects are marketed in the EU:

- 1) whole, as is, for snacks or incorporated in preparations such as biscuits,
- 2) as ingredients, i.e. in the form of flour, after grinding. This presentation ensures that:
 - > the insect is no longer recognisable by the consumer.
 - the hard chitinous parts are ground and incorporated. The powders obtained may or may not be sieved/fractionated, with successive grindings.

It should be noted that grinding <u>without fractionation</u> is not generally regarded, within the meaning of the regulations, as a "processing" operation aiming to extract a specific component. Foods described as "unprocessed" can indeed be simply ground. For edible insects that have undergone dehydration and cooking, they are in any event classified in the category of processed foods, and the final grinding does not change this classification in any way. However it may be considered that sieving after grinding, to eliminate certain fractions and therefore concentrate the consumed part in other fractions, constitutes an operation to extract "products derived from insects". This point may seem anecdotal but could become important with regard to the regulatory status of food products consisting fully or partly of edible insects, in the light of the definitions established in the European Commission regulation on novel foods.

The sensitivity to oxidation is increased by grinding, and the flours obtained must be packaged with care.

In general, it should be recalled that, as with other foods of animal or plant origin, edible insects can become unfit for human consumption following unsuitable preservation.

4.2.5. Food applications

In general, insects used in human food are grilled, fried or boiled. Some traditional modes of consumption in use in Asia, South America or Africa have not been included here, because it is unlikely that they would be offered to European consumers⁷.

The incorporation of insects and insect products in animal feed could be considered without prior heat-treatment. They must then be consumed quickly after harvest. If they are transported to be sold a long way from the production sites, conditions must ensure the preservation of their nutritional quality and safety.

4.3. Analysis of the hazards associated with the consumption of insects

The health hazards associated with insects or insect products can be of two main types:

- Specific to the species: presence of microbial hazards or hazards of microbial origin, foreign bodies, toxic substances (intrinsic or bio-accumulated), antinutritional substances or allergens;

⁷ In these geographical areas, some species are prepared with complex manual interventions, such as in particular removing the intestine and/or the rostrums, the hard elytra, or other parts: antennas, legs, wings and other hard or indigestible parts from each animal, which obviously requires skilled labour. These types of products and preparations are not currently offered in Europe.

- Related to rearing (food, veterinary medicines) or processing practices, or preservation and transport conditions.

The hazards presented here may relate to both human food and animal feed, and mainly describe the lack of knowledge that would enable an exhaustive analysis of the hazards.

The hazard analysis was conducted according to the literature existing to date and to what were considered to be reasonably predictable conditions for the distribution/consumption of insects in France today.

4.3.1. Chemical hazards

a. Toxic substances

The chemical hazards mainly result from substances manufactured by the insect itself or substances accumulated by the insect *via* its environment or food. Not all insect species are therefore edible as is, or may only be edible at the larval phase and not the adult state, or vice versa, and may remain inedible even under managed rearing conditions and after processing by cooking and drying.

Certain categories of insects synthesise toxic substances for defence or repellent purposes, of endocrine origin (for example the formic acid secreted by ants or the quinones emitted in the form of a 100°C jet by bombardier beetles) or non-glandular origin (for example some butterflies belonging to the *Papilionoidea* superfamily are able to synthesise *de novo* cyanogenic toxic compounds such as linamarin or lotaustralin from, respectively, valine and isoleucine) (Eisner 1970; Zagrobelny, Bak *et al.* 2004). Some larvae have also developed a self-defence system with a process of melanisation, by which they blacken and become unfit for consumption because of the appearance of toxic products (e.g. larvae of *Galleria mellonella* infected by a fungus (Slepneva, Komarov *et al.* 2003)).

A distinction is made between:

- insects known as phanerotoxic, with external venomous features such as the stings of Hymenoptera (including bees, wasps and ants), the piercing mouthparts of Hemiptera (including true bugs, cochineals, etc.) or the urticating bristles of some Lepidoptera. Envenomation by phanerotoxic insects occurs by the inoculation of venom (Hymenoptera) or by contact with urticant products (the pine processionary caterpillar that causes inflammatory reactions (Pouvreau 1999)). However, envenomation may also concern the dietary route: this is the case with larvae of *Trogoderma* spp, from the order Coleoptera, which are capable of causing intestinal trauma by envenomation due to the bristles found on the insect. A case of ulcerative colitis was reported in a child aged 4 months fed grain infested with *Trogoderma* (Okumura 1967). However, to limit the toxic risks of ingestion of venom, the consumption of larvae, which have no sting, will generally be preferable to that of adult insects.
- so-called cryptotoxic insects, which are able to store and/or synthesise toxic chemical elements, and whose toxicity only appears if the insect is consumed. They contain toxic substances that they have either synthesised themselves, or accumulated from the plants they consume. Phytophagous insects can accumulate plant toxins thereby acquiring the same toxic properties as the host plants (Berenbaum 1993). They may, in turn, possibly

develop adaptive strategies such as detoxification, excretion and bioaccumulation, in order to avoid any intoxication. These phytotoxins are secondary metabolites synthesised by vascular plants as active defence mechanisms; they belong to very varied classes of compounds: alkaloids, cardenolides, glucosinolates, cucurbitacins, or phenolic or cyanogenic compounds (Bennett and Wallsgrove 1994; Nishida 2002). Oligophagous insects (those feeding on a limited number of plant species) accumulate more phytotoxins than polyphagous insects. Variations in accumulation have also been observed with the insect's stage of development and its physiological state (Bennett and Wallsgrove 1994; Berenbaum 1993). It is therefore necessary to feed the farmed insects only with plants suited to their metabolism, avoiding any production or bioaccumulation of secondary metabolites that are toxic to vertebrates. It is necessary to screen plant and insect components to detect the presence and concentration of toxic molecules in order to assess whether the plant can be used as food for the insect and whether the insect can be regarded as edible or not, whether by humans or by animals. Among the insects that can be reared, Lepidoptera are known to bioaccumulate toxic substances very easily (Zagrobelny, Bak et al. 2004).

In addition, insects can accumulate undesirable substances found in their environment or feed, such as pesticides, persistent organic pollutants or heavy metals. There are few quantitative and qualitative data on the accumulation of pesticides. Only one study by Saeed, Abu Dagga et al. (1993) demonstrated that locusts are "efficient" bioaccumulators of insecticides. In farms therefore, the levels of pesticides in the feed offered to the insects must be strictly monitored because their accumulation is likely to present risks to human food and animal feed since these compounds are generally not eliminated by heat treatments. The same is true for persistent organic pollutants. For example, Gaylor, Harvey et al. (2012) demonstrated the capacity of domestic crickets to bioaccumulate polybrominated diphenyl ethers found in polyurethane foams. And lastly, several studies have demonstrated the presence of metal trace elements in insects: cadmium in the larvae of Tenebrio molitor (Vijver, Jager et al. 2003), lead in grilled crickets in Mexico (Handley, Hall et al. 2007), and arsenic in a moth consumed by Aborigines in Australia (Green, Broome et al. 2001). More recently, Zhuang, Zou et al. (2009) showed a slight bioaccumulation of metal trace elements (lead, zinc, copper and cadmium) between the different trophic levels of the soil-plant-insectchicken food chain, although these elements were relatively effectively eliminated in the excreta of the insects.

Like existing conventional livestock farms, the use of veterinary medicines should be planned in insect farms to reduce the mortality especially associated with bacterial or parasitic infections. There are very few reports in the literature of the presence of veterinary drug residues in insect tissues. A study of a disease in silkworms (*Bombyx mori*) refers to the use of chloramphenicol, a broad-spectrum antibiotic whose use is prohibited in animal production (Regulation (EU) No. 37/2010 2010). Cappellozza, Saviane *et al.* (2011) showed that this antibiotic, administered via feed, was not inactivated in the digestive tract of the silkworm.

The methods, processes and equipment used in the food production chain may be responsible for the transfer of contaminants from food contact materials (FCMs), and the production of newlyformed substances from compounds found in the raw material including additives or processing aids following various treatments. Nibbling of plastic supports must be avoided, in particular by

larvae, and rearing practices must be controlled by good hygiene practices and a hazard analysis during primary production to avoid levels of active chemical substances or exogenous contamination exceeding regulatory thresholds. The conditions of preservation and transport as well as the consumption practices (raw, cooked, grilled, etc.) may also be responsible for health risks associated with toxic substances (products of the Maillard reaction, newly-formed products).

b. Antinutritional factors

The presence of antinutritional substances has also already been demonstrated in some insect species. This problem is of particular importance among people whose diets are deficient in vitamins or any other important nutrient (Belluco, Losasso *et al.* 2013). The major antinutritional factors identified among insects are:

- phytic acid, which decreases the bioavailability of phosphorus by complexing it into phytate,
- oxalates which, when absorbed in large quantities, cause irritation of the digestive tract, blood circulation disorders and kidney damage,
- hydrocyanic acid, highly toxic because it causes anoxia,
- tannins, toxic at high doses as they precipitate proteins,
- thiaminase, which causes a deficiency in vitamin B1, and was responsible, for more than 40 years, for a major seasonal ataxic syndrome in Nigeria (Nishimune, Watanabe *et al.* 2000).

The composition in mg/100g of dry weight of the first four substances for a few insect species (or orders for undetermined species) is provided in Annex 2.

Animals are susceptible to these antinutritional factors. Before any use of insects as feed, it is therefore necessary to determine whether these substances are present, and if so at what concentrations. Where necessary, methods will have to be found to eliminate these substances, by heating or extrusion for example, when they are sensitive to these methods.

In addition, chitin, a constituent of the insect exoskeleton and chitosan, one of its derivatives, can be regarded as antinutritional factors. Indeed, apart from the fact that chitin is not digested or is poorly digested by animals whose digestive tracts often lack chitinase (in whom it causes constipation phenomena that may extend to intestinal obstruction), chitin and chitosan can bind to lipids and form gels that capture certain vitamins and minerals, thus reducing their bioavailability. This strong ability to bind to lipids (approximately 15 times the weight of the chitosan), is currently being studied in research on obesity.

4.3.2. Physical hazards

Edible insects are usually eaten whole, or after preparation to remove certain hard parts whose consumption is not desired (elytra, rostrums, wings, etc.). Edible insects are not especially vectors of physical hazards in the classic sense (dense contaminants or foreign bodies). During their processing, they may be re-contaminated by foreign bodies from the processes, as with any other processed food.

Dehydrated insects consumed whole contain hard parts that may present a particular hazard. Similarly, insects with a sting or a sharp rostrum may pose a specific hazard. These hazards related to the presence of foreign matter must be taken into account. Consumers should be informed of the presence of these hard parts naturally found in the product. Thus, the FASFC

stated in its opinion⁸ that *"it is therefore strongly advised to indicate where appropriate on the product label that the insect's legs and wings must be removed before consumption"*. The insect products presented ground and in the form of flour are not vectors of specific foreign bodies, other than those generated by the processes.

4.3.3. The allergen hazard

The risk of food allergies is one of the most predictable due to the existence of allergens (panallergens) common to arthropods, arachnids (mites, spiders, scorpions), crustaceans (lobster, shrimp, crabs) and insects. Similarly, mollusc and helminth allergens are often very similar to those of insects and can give rise to cross-reactions and/or allergies (Barre, Caze-Subra *et al.* 2014).

Food allergy phenomena in livestock animals are poorly documented but the possibility of crossallergies cannot be excluded, because meal made from arthropods is a potential ingredient in their diets; it is used, for example as a protein supplement and appetite stimulant in feed for farmed fish.

a. Allergic reactions in humans reported in the literature

Several cases of allergy have been reported among laboratory staff assigned to the maintenance of insect farms. They essentially concern respiratory disorders (cough, rhinitis, dyspnoea, bronchitis, asthma) but also skin manifestations (itching, pruritus). These allergic reactions are attributed to both aero-allergens and contact allergens. Other occupational allergies have been identified among farmers, agricultural workers and bakers, in this last case, due to insects contaminating flour.

Several cases of food allergy due to ingestion of insects have been reported in the literature. The insects incriminated were the mealworm (*Tenebrio molitor*), superworm (*Zophobas morio*), silkworm (*Bombyx mori*), red palm weevil (*Rhynchophorus ferrugineus*) and the mopane worm (*Gonimbrasia belina*). "Pancake syndrome" is a food anaphylaxis resulting from accidental ingestion of dust mites contaminating cereal flours (Sanchez-Borges, Suarez-Chacon *et al.* 2005).

A case of anaphylactic shock in a French tourist due to the consumption of silkworm pupae (*Bombyx mori*) was reported by Ji, Zhan *et al.* (2008). These authors mention different publications (in Chinese), that refer to 13 anaphylactic reactions due to the ingestion of silkworm pupae fried in oil. They believe that in China, each year, more than a thousand anaphylactic reactions are recorded after consumption of these roasted pupae. These figures suggest that anaphylactic reactions devoted to this subject. Several cases of cross-reaction between the caterpillar fungus used in traditional Chinese medicine (*Ophiocordyceps sinensis*) and silkworm pupae (*Bombyx mori*), have even been reported by Choi, Shin *et al.* (2010). The allergen responsible was not identified.

In 2012, a severe case of food allergy (with hospitalisation) to the larvae of the red palm weevil (*Rhynchophorus ferrugineus*) was reported in Malaysia in a Chinese tourist who consumed about twenty roasted larvae (Yew and Kok 2012).

⁸ Opinion of the High Council for Health and of the Belgian Federal Agency for the Safety of the Food Chain (FASFC) in answer to the question "does consumption of insects present health risks or not?" <u>http://www.health.belgium.be/eportal/Aboutus/relatedinstitutions/SuperiorHealthCouncil/ 19099435_FR</u>

b. Allergens of edible insects

Overall, insect allergens remain poorly understood, even though some of the few insects responsible for severe allergic reactions have been well studied and characterised. This is the case with cockroach allergens and allergens in Hymenoptera venom (bees, wasps, hornets). Edible insect allergens have only been subjected to a very limited number of studies (silkworm (Barre, Caze-Subra *et al.* 2014)). With respect to the antigens contained in the venom of phanerotoxic insects, the associated risk for animals or humans could be excluded by choosing species or stages of development (larvae in the case of Hymenoptera) lacking stings (Belluco, Losasso *et al.* 2013).

Most of the insect allergens correspond to ubiquitous proteins or pan-allergens, likely to cause cross-reactions. This is the case with muscle proteins (actin, myosin, tropomyosin, troponin C), cellular proteins (tubulins), circulating proteins (hemocyanins, defensins) and many proteins with enzymatic properties (α -amylase, arginine kinase, glutathione S-transferase, triosephosphate isomerase, trypsin).

Chitin, a fundamental constituent of the exoskeleton of arthropods (cuticle of mites and insects, carapace of crustaceans), of various mollusc organs (radula of gastropods, beak of the octopus), of the cell walls of mould and the tegument of helminths, is also regarded as an allergen but its effects on the immune system are complex. In insects, it acts on innate immunity but its effects differ depending on the size of the *N*-acetylglucosamine chains (chitosans) of which it is constituted. It can have immunostimulant effects or on the contrary, can decrease the allergic response (Lee, Simpson *et al.* 2008; Muzzarelli 2010). An EFSA opinion states the absence of risk to humans, under the recommended conditions of use (between 2 and 5g/day), of a food supplement containing 90% chitin-glucan (EFSA 2010). The high levels of chitin in edible insects can however pose a problem of digestibility, because the chitinases identified in gastric secretions do not seem active enough to hydrolyse this polymer.

The characteristics of *N*-glycan fucosylation in insects create new immunochemical specificities or glycotopes, which can be recognised by allergy sufferers in the same way as protein epitopes. For this reason, the use of therapeutic recombinant proteins expressed by baculoviruses may be advised against. These Fuc α 1-3GlcNAc groups create new glycotopes that allergy sufferers can recognise.

c. Cross reactions and/or allergies

The more or less close phylogenetic relationships that exist between the different phyla of arthropods fully explain these sequence and structure homologies, which are themselves responsible for the existence of common B epitopes in certain allergens (pan-allergens), which can cause cross-reactivity/allergies between edible insects and other arthropods, mites (Arachnids), crustaceans and inedible insects (cockroaches).

Examples of pan-allergens involved in cross-reactions between insects and crustaceans have been reported. These few examples give substance to the possibility of cross-reactivity and/or allergies between edible insects and other arthropods such as mites, cockroaches, shrimp,

molluscs and even nematodes. The consumption of insects by individuals with allergies to dust mites or shrimp could very well trigger allergic reactions attributable to this cross-reactivity.

In a recent study (Verhoeckx, van Broekhoven *et al.* 2013), the existence of cross-reactivity between dust mites (the tropomyosin "Der p 10" of *Dermatophagoides pteronyssinus*) and the mealworm (*Tenebrio molitor*) was demonstrated. This is based on pan-allergens and tropomyosin, but also on other allergens such as arginine kinase, triosephosphate isomerase and tubulins. The first two have been identified as the main proteins responsible for the cross-reaction. Tropomyosin belongs to a family of highly conserved proteins with multiple isoforms (due to variations in a few amino acids), that are found both in muscle and non-muscle cells of all the species in the animal kingdom (Leung, Wing Kuen *et al.* (1996); Reese, Ayuso *et al.* (1999) cited in Belluco, Losasso *et al.* (2013)). Tropomyosin is a thermostable allergen, from 32 to 39 kDa, consisting of two alpha helices wound around each another, giving the protein a helical structure (Metz-Favre, Rame *et al.* 2009). For its part, arginine kinase is an enzyme often found in invertebrates and an allergic cross-reaction is already known between different crustaceans, mites, *Plodia interpunctella* (Lepidoptera: Pyralidae), *Bombyx mori, Blatella germanica* (Blattodea - Blattidae) and *Periplaneta americana* (Blattodea - Blattidae) (Liu, Xia *et al.* 2009; Verhoeckx, van Broekhoven *et al.* 2013).

The possibility of cross-reactions between edible insects and other groups of arthropods (mites, crustaceans), molluscs and nematodes, deserves to be studied on a larger scale.

The existence of cross-reactivity does not automatically imply the existence of cross-allergies between edible insects and other arthropods, molluscs and helminths. Many cross-reactions have no clinical significance but they should encourage caution in the practice of entomophagy. The consumption of insects by people who are allergic to other arthropods, especially crustaceans, is expected to trigger cross-reactions (cross-allergies?) related to pan-allergens. In this event, it is prudent to recommend that these subjects avoid consumption of edible insects or products containing them.

In France, if there were a real allergic risk linked to cross-reactions and/or cross-allergies, it would not *a priori* be negligible, as suggested by a simple estimate:

- It is estimated that food allergies affect 3.5% of the French population, i.e. one person in 30 (2,300,000/65,500,000), of all ages.

- It is estimated that allergies to crustaceans and shellfish affect 2% of children and 3% of adults suffering from food allergies, i.e. 5% of these people (115,000), of all ages, i.e. one French person in 20 with food allergies (115,000/2,300,000) or one French person in 550 (0.1%) as a proportion of the total population (115,000/65,500,000).

- Around 100,000 people, or the population of an average French city, could therefore potentially be affected by these cross-reactions and/or cross-allergies.

In fact, this figure is very imprecise and should be significantly lower, because most crossreactions observed in food allergies do not lead to any clinical symptoms. But the hazard cannot be excluded at present, even though it cannot be estimated in the absence of any large-scale study.

4.3.4. Microbiological hazards

In general, compared to other foodstuffs of animal origin, there is a major shortage of information on microbiological hazards.

a. Parasitic hazards

The parasitic risks associated with insects are quite poorly documented and the few studies published mainly concern the risks in humans.

Insects, particularly those considered for mass production, can be carriers of parasites and this hazard must be considered seriously. Thus, parasites have been demonstrated in samples of insects in the framework of a study on intestinal parasitoses in South-East Asia (Chai, Shin *et al.* 2009). Mammals, birds and fish may constitute host reservoirs. Several types of parasitic infections in relation to the consumption of insects have been described:

- Parasitic infections to cercaria and metacercaria, via aquatic insects or insects living close to water, for which some species of birds and fish can be host reservoirs (Chai, Shin *et al.* 2009).
- Parasitic infections due to nematodes, especially *Gongylomena pulchrum*, a genus of nematodes from the *Spiruroidea* superfamily, for which Coleoptera and Blattodea are the intermediate hosts. Cases of zoonoses following consumption of such insects raw have been reported (Wilson, Lorente *et al.* 2001) and cannot be excluded in production livestock.
- Chagas disease, a parasitic disease that is rampant in rural areas of Central and South America, caused by *Trypanosoma cruzi*, which is transmitted by kissing bugs (bloodsucking bugs from the sub-family of *Triatominae*) via bites but also via droppings. Birds appear to be immunised against this parasite but many mammals can constitute host reservoirs (Pereira, Schmidt *et al.* 2010).
- Intestinal myiases caused by Diptera larvae including *Hermetia illucens*, the black soldier fly, which is reared for animal feed (Sehgal, Bhatti *et al.* 2002).
- Toxoplasmoses due to cockroaches and some Diptera (Graczyk, Knight *et al.* 2005).

The consumption of raw insects seems to have been linked with certain parasitic diseases in consumers. A better knowledge of the parasite/insect relationships would lead to the identification of good farming practices, or even targeted treatments during production. The adoption of deworming strategies, as well as good management practices for animals and rearing trays are needed to reduce the risk of contamination and reinfection by parasites. Certain modes of production include freeze-drying (without heat treatment). The effectiveness of this type of treatment on the parasites should be assessed, along with the effectiveness of freezing the insects. This risk is very probably less acute for heat-treated insects, even though in general, it would be wise to reassess certain scales, in the light of this matrix.

b. Viral hazards

There is no documentation on the viral risks associated with the handling or ingestion of insects.

c. Bacterial hazards and their toxins

Pathogenic bacteria of insects (entomopathogenic) are regarded as harmless to animals and humans due to the fact that the hosts are so phylogenetically different (van Huis 2013). The bacterial risks associated with insects will therefore mainly be due to the carriage (natural or accidental) of proven bacterial hazards (and their toxins) for humans and animals, the rearing conditions (substrates and feed), handling, processing and preservation. Examples of microbial

deterioration (defective preservation) of prepared insects show that they can constitute an environment compatible with the survival/growth of bacteria. Little information is available on the compatibility of this environment with toxinogenesis. Only a few cases of botulism in Africa have been reported after consumption of insects (Schabel 2010).

The bacterial agents that can potentially be transmitted via consumption of insects seem:

- to be either related to the intrinsic flora of insects (digestive tube and other anatomical compartments),
- or to have an extrinsic origin related to the environment and rearing conditions.

Bacilli such as those causing anthrax (*B. anthracis*) or food poisoning (*B. cereus*) can be transmitted by insects that have themselves been contaminated by soil used as a farming substrate. Spores from microorganisms may be found on the cuticle of insects and thus be consumed by animals or humans (van Huis 2013).

A very few publications mention the lack of positive results following screening for a few "major" pathogens (*Salmonella, L. monocytogenes, E. coli* and *C. perfringens*) for different categories of processed insects (most often cooked) (Alabi, Fievez *et al.* to be submitted; Giaccone 2005). Other work has enabled the isolation of pathogenic bacteria (*Staphylococcus aureus, Pseudomonas aeruginosa and B. cereus*) from *Oryctes monocerus*, a beetle commonly consumed in West Africa (Banjo, Lawal *et al.* 2006). However, analyses undertaken on several insects that could be farmed (the superworm *Zophobas morio*, the mealworm *Tenebrio molitor*, the greater wax moth *Galleria mellonella*, and the house cricket *Acheta domesticus*) have not revealed the presence of *Salmonella* or *L. monocytogenes* (Giaccone 2005; van Huis, van Itterbeeck *et al.* 2013).

The bacterial flora found in the different edible insects analysed are conventional, known food contaminants with different risks to animal and human health according to the species and probably the method of insect production.

d. Fungal hazards

Fungal flora and particularly species producing mycotoxins have been isolated from samples of insects in several studies. The most common isolates are *Aspergillus, Penicillium, Fusarium, Cladosporium* and *Phycomycetes* (Simpanya, Allotey *et al.* 2000) which come from firstly, initial contamination by leaves and soil and secondly, recontamination related to poor drying and storage conditions. Mycotoxins can be produced by strains of *Aspergillus, Penicillium* and *Fusarium,* even though this is rarely reported in studies. In 1996, researchers found levels of aflatoxins varying from 0 to 50 µg per kg of product in samples of the mopane worm *Imbrasia belina* (widely consumed in southern Africa) (Mpuchane, Taligoola *et al.* 1996). This can be a problem with regular consumption of these caterpillars.

e. Hazards associated with non-conventional transmissible agents (NCTA)

The possibility that ectoparasites can serve as a reservoir or vector for prions has been suggested in several publications (Lupi 2006; Wisniewski, Sigurdarson *et al.* 1996). Horizontal transmission of scrapie in small ruminants within infected herds is recognised and insects may play an active role in contamination. Bioassays have been performed in the laboratory from insect pupae (*Sarcophaga carnaria*) that had ingested infectious elements of central nervous systems. Hamsters

fed experimentally with these insects subsequently developed scrapie. Adult flies may also have the ability to express prion proteins (Lupi 2003; Post, Riesner *et al.* 1999). The risk related to NCTAs cannot therefore be ruled out.

f. Effectiveness of physical insect processing/preservation treatments on microbiological agents

Several physical treatments can be considered or applied to make insects edible, process and/or preserve them in order to consume them. These are often "conventional" heat treatments. To date, no information has been published on the inactivating effect of alternative physical treatments (high pressure, pulsed light, etc.). As a reminder, the use of ionising radiation at low doses is authorised for eradicating insects from fruits and seeds as part of pest control⁹. The influence of the food matrix on heat resistance is well known and has been described for a large number of food matrices common in Europe (protective effect of the food, acid pH-temperature synergy, etc.). This has often led to the development of reference heat treatments suited to the matrix. It seems important that our references be re-examined in light of the unusual matrix represented by insects. The great diversity of insect species that can be consumed, added to the diversity of the stages at which they can be consumed, and the different modes of preparation/consumption, all reinforce the need for such checks. The few studies available that focused on assessing the effect of heat treatments in insects on total bacterial flora, yeasts and moulds or specific flora, also concur. For van Huis et al. (2013), one way of eliminating possible insect contaminants is to cook (boil or roast) or pasteurise them. However, the counts performed still show relatively high numbers of cfu/g (10⁶ to 10^{7} /g in total flora) for cooked (boiled) products. It is easy to imagine that the total initial flora in the whole insects must be higher still. These results, which warrant consolidation, may nevertheless be very useful for qualifying or conducting a processing method or establishing a reference pasteurisation value (PV). Studies on the impact of several production, preservation and processing methods on the diversity and abundance of microorganisms found according to the candidate insects for the European market will also be needed.

4.4. Conclusions of the CES

Live and processed insects can be regarded as potential reservoirs and/or vectors of physical, chemical and biological agents (and their toxins), which may affect the health of humans and animals when consumed directly, or indirectly *via* livestock feed.

Hazards can occur either naturally (insect = primary reservoir of the biological hazard (digestive tube and other anatomical parts)), or from contamination during production, processing and preparation processes (rearing on a contaminated substrate, contaminated feed, insufficient inactivation treatment, defective preservation, transfer of contamination, etc.).

The health qualities of the insect considered as food must be of the same level as other foods. To date, the different production phases (rearing, preparation, packaging, preservation) for edible insects have been studied on a limited number of species, and additional targeted studies are necessary.

All the processes implemented to ensure food safety will have to guarantee that the objectives set by the regulations on food hygiene are met. Verification of these production conditions should also

⁹ lonising radiation is applied at doses that are insufficient for eliminating bacteria, and is only intended to ensure sterilisation of insect pests.

be proposed, as with products of animal origin, focusing on the nature and origin of the feed given to the insects, control of risks, and guarantee as to the biosafety of facilities. This verification of rearing, preparation and packaging of edible insects should apply to whole insects and to any derived products, insect meal in particular, likely to be included in the preparation of various food products.

Considering the very specific metabolism of insects, particular precautions must be taken in the rearing, preparation and marketing of insects for food, especially with analytical screening for any toxic substances in the finished products, on a case-by-case basis, as required by the pre-market authorisation of novel foods (Regulation (EC) No 258/97).

This work has helped highlight the great lack of scientific information that would facilitate effective risk assessment.

The Committee wishes to make the following remarks:

- The use of wild insects collected from the natural environment should be excluded, in favour of farmed insects with an assurance of traceability, and able to benefit from a full HACCP approach.
- Only certain species may be consumed. It would be advisable to establish preferentially (i) a positive list of species that have already been adequately assessed in order to facilitate their consumption; this list should not be definitive, and (ii) a "negative" list of species that have already been assessed, that present identified hazards and are prohibited for human consumption; this list may not be definitive if the appropriate inactivation treatments can be implemented. Species identified as likely to contain venom or toxic substances, at the stage at which they are harvested, should undergo specific analyses to ensure their safety after processing.
- The development of insect farming should be accompanied by the formulation of recommendations on good practices (rearing, transport and slaughter).
- The fasting of insects before slaughter, its effects on the digestive microflora and its impact in terms of health risks must be assessed.
- To avoid the bioaccumulation of toxic products in insects, feed should be strictly controlled, along with the quality of farming substrates (organic substrates in particular). A specific regulation should in particular be considered with regard to undesirable substances and maximum residue limits (MRLs) for plant protection products, veterinary medicinal products and crop inputs, due to specific metabolism and bioaccumulation phenomena for toxic substances.
- A full analysis of the hazards should be carried out for insects used in food, as recommended in the Regulation on novel foods (Regulation (EC) No 258/97). More detailed microbiological analyses, targeting the major pathogens (and their toxins) and a few well chosen indicators, possibly supplemented by genomic analyses to identify all the dominant microorganisms found in the insects, and the associated biological hazards, should be undertaken on a limited number of candidate insects for possible mass production.
- The major pathogens potentially found in the finished products should undergo analysis. In particular, the matrix-insect/toxinogenesis relationship for *S. aureus, B. cereus* and *C. botulinum* should be better understood. A better knowledge of parasitic infections associated with consumption of insects seems to be necessary. The same is true for prions, for which the

risk cannot be excluded. In addition, it is necessary to consider the viral hazard, which has not to date undergone study.

- The main chemical risks in the production of insects for food purposes seem to result from substances produced in sufficient quantity by certain species, above the thresholds of reactivity and/or health protection for humans, or substances accumulated during metabolism by the insect from its environment as part of the wide variability of dynamic interactions involving many species and eco-environmental factors. Toxicological studies should be performed on a selected panel of non-toxic species, such as those conducted by a Chinese team on proteins in silkworm pupae (Zhou and Han 2006). The processing methods can also be responsible for the emergence of newly-formed substances, and the risks associated with their presence must be assessed.
- The physical parts (wings, legs, rostrums, etc.) naturally found in insect products must be taken into account in the hazard analysis.
- The existence of numerous proteins common to edible insects, other arthropods (mites, crustaceans), molluscs, mould and helminths, could lead to cross-reactions with the IgE of subjects allergic to crustaceans and mites in particular. This demonstrates the importance of experimentally analysing the potential allergy risk in order to characterise the main allergens specific to a few insect species, clarify the resistance of these major allergens to thermal and digestive denaturation, study the evolution of these major allergens during prolonged preservation of edible insects, and verify the existence of cross-reactions. Furthermore, although the question is outside the scope of this Opinion, the CES wishes to emphasise that in view of the exposure of different sector participants to allergens, measures must be taken to prevent allergy risk in the workplace.
- In general, because the insect is an unusual food matrix, it should be studied in order to better qualify and, if necessary, adapt purifying practices associated with the processes of cooking, drying, refrigeration, freezing, heat treatment, etc. The satisfactory microbiological quality of prepared insects requires a heat treatment at least equivalent to pasteurisation, obtained by cooking and/or drying.
- Preservation techniques should be adapted to ensure management of the lifetimes of insects and products derived from insects. Dehydrated insects should be brought to a level of residual moisture compatible with their preservation, and wrapped in air-tight packaging, or packaged in a modified atmosphere, suitable for the stated durability date, taking into account the high levels of unsaturated fatty acids which make these products very sensitive to oxidation.
- For human consumption, in the absence of a guarantee of the microbiological safety of foods derived from insects, it is preferable to not eat them raw. The microbiological quality of these foods derived from insects should also be taken into account for animal feed.
- Adequate labelling should warn the consumer of the presence, in any form, of insects in food. More particularly, the consumer should be informed about the possible presence of the hard parts of insects (wings, legs, rostrums, etc.), naturally found in the product.
- Clear communication seems essential on the reasonably foreseeable misuses/improper uses of insects and products derived from insects; for example, the sale of insects or insect products intended for animal feed and diverted to human food.

5. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the CES BIORISK's conclusions.

Entomophagy is a very widespread practice in some parts of the world (Africa, Asia, Latin America) where it can form part of the traditional food culture. The FAO estimates that "insects form part of the traditional diets of at least 2 billion people" in the world and has spoken out in favour of developing the rearing of insects on a large scale to meet growing concerns about food security and the supply of protein. In Europe, this practice seems to be growing in popularity and several industrial projects and research programmes are supporting this emerging sector, despite the regulations in force (currently evolving rapidly) raising many questions.

This context led ANSES to conduct a review of scientific knowledge on the subject, with particular emphasis on documentation of the potential health risks associated with the consumption of insects and insect products, in both animal feed and human food.

This work has highlighted the lack of scientific data available on subjects related to this study, such as the environmental impact of insect production compared to other protein sources (especially on the subjects of the ecological footprint and energy cost) and the nutritional value of the different insect species and insect products. This observation is also valid in relation to the topics at the heart of this work, such as the specific hazards related to insects and safety in consumer countries, which to this day seems to be demonstrated more by a history of consumption than by any scientific risk assessment studies.

This study has thus highlighted the need for research that would enable a full assessment of the health risks associated with the consumption of insects. Indeed, like all foods, insects may present hazards that need to be controlled by establishing specific standards in order to reduce the potential risks associated with the consumption of these products.

These hazards are mainly related to:

- endogenous substances specific to certain categories of insects possessing venom or antinutritional factors;
- the rearing and production conditions for which a specific framework should be defined to guarantee the control of health risks;
- specific sensitivities in certain consumers, taking into account the presence in the insects of pan-allergens common to all arthropods (mites, crustaceans, molluscs, etc.).

ANSES thus recommends:

- intensifying the research effort on these topics;
- establishing, at EU level, positive and negative lists of the different insect species and stages of development that may or may not be consumed;
- exploring on a scientific level the question of animal welfare for these categories of invertebrates;
- defining a specific framework for rearing and production conditions for insects and their products to guarantee the control of health risks;

• establishing measures for the prevention of allergy risk both for consumers and in the workplace.

Pending the establishment of these specific standards and a suitable framework, ANSES calls for caution among consumers, especially those susceptible to food allergies.

Apart from the challenges of expert appraisal specifically associated with questions of assessing the health risks and nutritional benefits related to the consumption of insects, ANSES emphasises the major challenges surrounding knowledge related to the social acceptability of these new foods or the associated challenges of development and environmental impact.

Marc Mortureux

KEYWORDS

Insects, entomophagy, food, feed, health risks, allergy.

REFERENCES

Adeduntan (2005) Nutritional and antinutritional characteristics of some insects foraging in Akure forest reserve Ondo State, Nigeria. *Journal of Food Technology* **3(4)**, p. 563-567.

Adesina AJ (2012) Proximate and anti-nutritional composition of two common edible insects: yam beetle (Heteroligus meles) and palm weevil (Rhynchophorus phoenicis). *Elixir Food Science* **48**.

Alabi T, Fievez T, Jonas M, Blecker C, Danthine S, Caparros R, Haubruge E, Francis F (*A soumettre*) Effect of sanitation treatment on the microbiological quality and nutritional value of edible insects.

Banjo AD, Lawal OA, Adeyemi AI (2006) The Microbial Fauna Associated with the Larvae of *Oryctes monocerus Journal of Applied Sciences Research* **2**(11), 837-843.

Barre A, Caze-Subra S, Gironde C, Bienvenu F, Bienvenu J, Rougé P (2014) Entomophagie et risque allergique. *Revue Française d'Allergologie* **In press**.

Bednářová M, Borkovcová M, Komprda T (2014) Purine derivate content and amino acid profile in larval stages of three edible insects. *Journal of the Science of Food and Agriculture* **94**(1), 71-76.

Belluco S, Losasso C, Maggioletti M, Alonzi CC, Paoletti MG, Ricci A (2013) Edible insects in a food safety and nutritional perspective: A critical review. *Comprehensive Reviews in Food Science and Food Safety* **12**(3), 296-313.

Bennett RN, Wallsgrove RM (1994) Transley Review No.72. Secondary metabolites in plant defence mechanisms. *New Phytologist* **127**(4), 617-633.

Berenbaum MR (1993) Sequestred Plant Toxins and Insect Palatability. *The Food Insect Newsletter* **6**(3), 1-12.

Bukkens SGF (1997) The nutritional value of edible insects. *Ecology of Food Nutrition* **36**(2-4), 287-319.

Cappellozza S, Saviane A, Tettamanti G, Squadrin M, Vendramin E, Paolucci P, Franzetti E, Squartini A (2011) Identification of Enterococcus mundtii as a pathogenic agent involved in the "flacherie" disease in Bombyx mori L. larvae reared on artificial diet. *J Invertebr Pathol* **106**(3), 386-93.

Cardon D (2003) 'Le Monde des teintures naturelles.' (Belin: Paris) 586

Chai JY, Shin EH, Lee SH, Rim HJ (2009) Foodborne intestinal flukes in Southeast Asia. *Korean Journal of Parasitology* **47**(SUPPL.), S69-S102.

Chen X, Feng Y, Chen Z (2009) Common edible insects and their utilization in China: INVITED REVIEW. *Entomological Research* **39**(5), 299-303.

Choi GS, Shin YS, Kim JE, Ye YM, Park HS (2010) Five cases of food allergy to vegetable worm (Cordyceps sinensis) showing cross-reactivity with silkworm pupae. *Allergy: European Journal of Allergy and Clinical Immunology* **65**(9), 1196-1197.

COM (2007) 872 (2007) Proposal for a Regulation COM(2007) 872 final of the European Parliament and of the Council, 2007. Novel foods. *Official Journal of the European Communities*.

Comby B (1990) 'Délicieux insectes. Les protéines du futur.' (Paris)

Dalgaard R, Schmidt J, Halberg N, Christensen P, Thrane M, Pengue WA (2007) LCA of soybean meal. *The International Journal of Life Cycle Assessment* **13**(3), 240-254.

de Vries M, de Boer IJM (2010) Comparing environmental impacts for livestock products: A review of life cycle assessments. *Livestock Science* **128**(1-3), 1-11.

DeFoliart GR (1991) Insect fatty acids: similar to those of poultry and fish in their degree of unsaturation, but higher in the polyunsaturates. *The Food Insect Newsletter* 4(1), 1-8.

Defoliart GR (1995) Edible insects as minilivestock. *Biodiversity and Conservation* **4**(3), 306-321.

Directive 2002/32/EC (2002) Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed.

Durst P, Shono K (2010) Edible forest insects: exploring new horizons and traditional practice. In 'Edible Forest Insect: Human Bite Back. Proceedings of a workshop on Asia-Pacific resources and their potential for development.' (Eds Durst PB, Johnson DV, Leslie RN and S K): Bangkok, Thailand)

EFSA (2010) EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the safety of 'Chitin-glucan' as a Novel Food ingredient. EFSA Journal 2010; Available online: <u>www.efsa.europa.eu/efsajournal</u>. 17 pp.

Eisner T (1970) Chemical Defense against Predation in Arthropods. In 'Chemical Ecology.' (Eds E Sondheimer and JB Simeone). (Academic Press Inc.: New York)

Ekop EA, Udoh AI, Akpan PE (2010) Proximate and anti-nutrient composition of four edible insects in Akwa Ibom State, Nigeria. *World Journal of Applied Science and Technology* **2**, p. 224-231.

FAO (2004) Agriculture, Food & Water.

FAO (2009) How to feed the world in 2050? Food and Agriculture Organization of the United Nations (FAO), Rome.

http://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050. pdf)

FAO (2011) 'Summary report: State of the world's land and water resources for food and agriculture.' (Rome)

Feedipedia (2014).

Gaylor MO, Harvey E, Hale RC (2012) House crickets can accumulate polybrominated diphenyl ethers (PBDEs) directly from polyurethane foam common in consumer products. *Chemosphere* **86**(5), 500-505.

Gerber PJ, Steinfeld H, Henderson B, Mottet A, Opio C, Dijkman J, Falcucci A, Tempio G (2013) Tackling climate change through livestock - A global assessment of emissions and mitigation opportunities. *Food and Agriculture Organization of the United Nations (FAO), Rome.* Giaccone V (2005) Hygiene and health features of "minilivestock". In 'Ecological implications of minilivestock. Potential of rodents, frogs, snails, and insects.' Ed. MG Paoletti) pp. 579–598. (Science Publishers: New Hampshire)

Gracer D (2010) Filling the plates: serving insects to the public in the United States. Eds: Durst, P. B.; Johnson, D. V.; Leslie, R. N.; Shono, K.: Forest insects as food: humans bite back. Proceedings of a workshop on Asia-Pacific resources and their potential for development, Chiang Mai, Thailand, 19-21 February, 2008, 2010, pp 217-220.

Graczyk TK, Knight R, Tamang L (2005) Mechanical transmission of human protozoan parasites by insects. *Clinical Microbiology Reviews* **18**(1), 128-132.

Green K, Broome L, Heinze D, Johnston S (2001) Long Distance Transport of Arsenic by Migrating Bogong Moths from Agricultural Lowlands to Mountain Ecosystems. *The Victorian Naturalist* **118**(4), 112-116.

Hackstein JH, Stumm CK (1994) Methane production in terrestrial arthropods. *Proc Natl Acad Sci U S A* **91**(12), 5441-5.

Haldar P, Das A, Gupta RK (1999) A laboratory based study on farming of an Indian grasshopper *Oxya fuscovittata* Marschall Orthoptera: Acrididae. *Journal of Orthoptera Research* **8**, 93-97.

Handley MA, Hall C, Sanford E, Diaz E, Gonzalez-Mendez E, Drace K, Wilson R, Villalobos M, Croughan M (2007) Globalization, binational communities, and imported food risks: results of an outbreak investigation of lead poisoning in Monterey County, California. *Am J Public Health* **97**(5), 900-6.

Ji KM, Zhan ZK, Chen JJ, Liu ZG (2008) Anaphylactic shock caused by silkworm pupa consumption in China. *Allergy* **63**(10), 1407-1408.

Kiuchi M, Tamaki Y (1990) Future of edible insects. Farming Japan 24, 37-41.

Klunder HC, Wolkers-Rooijackers J, Korpela JM, Nout MJR (2012) Microbiological aspects of processing and storage of edible insects. *Food Control* **26**(2), 628-631.

Lee KP, Simpson SJ, Wilson K (2008) Dietary protein-quality influences melanization and immune function in an insect. *Functional Ecology* **22**(6), 1052-1061.

Leung PSC, Wing Kuen C, Duffey S, Hoi Shan K, Gershwin ME, Ka Hou C (1996) IgE reactivity against a cross-reactivity allergen in crustacea and mollusca: Evidence for tropomyosin as the common allergen. *Journal of Allergy and Clinical Immunology* **98**(5), 954-961.

Li L, Zhao Z, Liu H (2013) Feasibility of feeding yellow mealworm (Tenebrio molitor L.) in bioregenerative life support systems as a source of animal protein for humans. *Acta Astronautica* **92**(1), 103-109.

Liu Z, Xia L, Wu Y, Xia Q, Chen J, Roux KH (2009) Identification and characterization of an arginine kinase as a major allergen from silkworm (Bombyx mori) larvae. *Int Arch Allergy Immunol* **150**(1), 8-14.

Lupi O (2003) Could ectoparasites act as vectors for prion diseases? Int J Dermatol 42(6), 425-9.

Lupi O (2006) Myiasis as a risk factor for prion diseases in humans. *J Eur Acad Dermatol Venereol* **20**(9), 1037-45.

Makkar HPS, Tran G, Heuzé V, Ankers P (2014) State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology* **197**(0), 1-33.

Metz-Favre C, Rame JM, Pauli G, de Blay F (2009) Tropomyosin: Pan-allergen. *La tropomyosine : un pan-allergène* **49**(5), 420-426.

Mignon J (2002) L'entomophagie : une question de culture ? *Tropicultura* **20**(3), 15-155.

Mpuchane S, Taligoola H, Gashe B (1996) Fungi associated with Imbrasia belina, an edible caterpillar. *Botswana Notes and Records* **28**, 193-197.

Muzzarelli R (2010) Chitins and Chitosans as Immunoadjuvants and Non-Allergenic Drug Carriers. *Marine Drugs* **8**(2), 292-312.

Nakagaki BJ, Defoliart GR (1991) Comparison of Diets for Mass-Rearing Acheta domesticus (Orthoptera: Gryllidae) as a Novelty Food, and Comparison of Food Conversion Efficiency with Values Reported for Livestock. *Journal of Economic Entomology* **84**(3), 891-896.

Nishida R (2002) Sequestration of defensive substances from plants by Lepidoptera. In. Vol. 47'. pp. 57-92)

Nishimune T, Watanabe Y, Okazaki H, Akai H (2000) Thiamin is decomposed due to *Anaphe* spp. entomophagy in seasonal ataxia patients in Nigeria. *Journal of Nutrition* **130**(6), 1625-1628.

Okumura GT (1967) A report of canthariasis and allergy caused by Trogoderma. *Californian Vector Views* **14**(3), 19-22.

Omotoso OT (2006) Nutritional quality, functional properties and anti-nutrient compositions of the larva of Cirina forda (Westwood) (Lepidoptera: Saturniidae). *Journal of Zhejiang University Science* **7(1)**, p. 51-55.

Oonincx, van Itterbeeck, Heetkamp, van den Brand, van Loon, van Huis (2010) An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PLoS ONE* **5**(12).

Oonincx DGAB, de Boer IJM (2012) Environmental Impact of the Production of Mealworms as a Protein Source for Humans – A Life Cycle Assessment. *PLoS ONE* **7**(12), e51145.

Pennino M, Dierenfeld ES, Behler JL (1991) Retinol, α-tocopherol and proximate nutrient composition of invertebrates used as feed. *International Zoo Yearbook* **30**(1), 143-149.

Pereira KS, Schmidt FL, Barbosa RL, Guaraldo AM, Franco RM, Dias VL, Passos LA (2010) Transmission of chagas disease (American trypanosomiasis) by food. *Adv Food Nutr Res* **59**, 63-85.

Post K, Riesner D, Walldorf V, Mehlhorn H (1999) Fly larvae and pupae as vectors for scrapie. *Lancet* **354**(9194), 1969-70.

Pouvreau A (1999) Les insectes venimeux urticants. *INSECTES* **114**(5), 9-12.

Ramos-Elorduy J (1997) Insects: A sustainable source of food? *Ecology of Food Nutrition* **36**(2-4), 247-276.

Ramos-Elorduy J (2009) Anthropo-entomophagy: Cultures, evolution and sustainability. *Entomological Research* **39**(5), 271-288.

Raubenheimer D, Rothman JM (2013) Nutritional ecology of entomophagy in humans and other primates. In. Vol. 58'. pp. 141-160)

Reese G, Ayuso R, Lehrer SB (1999) Tropomyosin: An invertebrate pan-allergen. *International Archives of Allergy and Immunology* **119**(4), 247-258.

Regulation (EU) No 56/2013 (2013) Commission Regulation (EU) No 56/2013 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies.

Regulation (EU) No 68/2013 (2013) Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of feed materials (1).

Regulation (EC) No 183/2005 (2005) Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. *Official Journal of the European Union* **31**.

Regulation (EC) No 258/97 (1997) Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients.

Regulation (EC) No 767/2009 (2009) Regulation (EC) No 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed, amending European Parliament and Council Regulation (EC) No 1831/2003 and repealing Council Directive 79/373/EEC, Commission Directive 80/511/EEC, Council Directives 82/471/EEC, 83/228/EEC, 93/74/EEC, 93/113/EC and 96/25/EC and Commission Decision 2004/217/EC.

Regulation (EC) No 852/2004 (2004) Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs

Regulation (EC) No 854/2004 (2004) Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption.

Regulation (EC) No 999/2001 (2001) Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies.

Regulation (EC) No 1069/2009 (2009) Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 Official Journal of the European Union **31**.

Regulation (EU) No 37/2010 (2010) Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.

Ritter (2010) Insect and Cholesterol. Food insects Newsl. 3(1), 1-6.

Rumpold BA, Schluter OK (2013a) Nutritional composition and safety aspects of edible insects. *Mol Nutr Food Res* **57**(5), 802-23.

Rumpold BA, Schlüter OK (2013) Nutritional composition and safety aspects of edible insects. *Molecular Nutrition and Food Research* **57**(5), 802-823.

Rumpold BA, Schlüter OK (2013b) Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science and Emerging Technologies* **17**, 1-11.

Saeed T, Abu Dagga F, Saraf M (1993) Analysis of residual pesticides present in edible locusts captured in Kuwait. *Arab Gulf Journal of Scientific Research* **11**(1), 1-5.

Sanchez-Borges M, Suarez-Chacon R, Capriles-Hulett A, Caballero-Fonseca F (2005) An update on oral anaphylaxis from mite ingestion. *Ann Allergy Asthma Immunol* **94**(2), 216-20; quiz 220-2, 306.

Schabel HG (2010) Forest insects as food: a global review. In 'Edible Forest Insect: Humans Bite Back. Proceedings of a workshop on Asia-Pacific resources and their potential for development.' (Eds Durst PB, Johnson DV, Leslie RN and S K): Bangkok, Thailand)

Schmidt JO, Buchmann SL (1992) 'Other products of the hive.' in 'The hive and the honey bee.' (Dadant and Sons: Hamilton, Illinois)

Sehgal R, Bhatti HP, Bhasin DK, Sood AK, Nada R, Malla N, Singh K (2002) Intestinal myiasis due to Musca domestica: a report of two cases. *Jpn J Infect Dis* **55**(6), 191-3.

Siemianowska E, Kosewska A, Aljewicz M, Skibniewska KA, Polak-Juszczak L, Jarocki A, Jędras M (2013) Larvae of mealworm (*Tenebrio molitor* L.) as European novel food. *Agricultural Sciences* **04**(06), 287-291.

Simpanya MF, Allotey J, Mpuchane SF (2000) A mycological investigation of phane, an edible caterpillar of an emperor moth, Imbrasia belina. *J Food Prot* **63**(1), 137-40.

Sirimungkararat S, Saksirirat W, Nopparat T, Natongkham A (2008) Edible products from eri silkworm (*Samia ricini* D.) and mulberry silkworm (*Bombyx mori* L.) in Thailand. *Proceedings of a Workshop on Asia-Pacific Resources and Their Potential for Development. RAP Publication 2010/02* **19-21**, 189–200.

Slepneva IA, Komarov DA, Glupov VV, Serebrov VV, Khramtsov VV (2003) Influence of fungal infection on the DOPA-semiquinone and DOPA-quinone production in haemolymph of Galleriamellonella larvae. *Biochem Biophys Res Commun* **300**(1), 188-91. [In eng]

Srivastava SK, Babu N, Pandey H (2009) Traditional insect bioprospecting - As human food and medicine. *Indian Journal of Traditional Knowledge* **8**(4), 485-494.

Steinfeld H (2006) 'Livestock's long shadow: environmental issues and options.' (FAO: Rome)

van Huis A (2010) Opinion: Bugs can solve food crisis. The Scientist - Magazine of the Life Sciences (Vol.).

van Huis A (2013) Potential of insects as food and feed in assuring food security. 58, 563-583.

van Huis A, van Itterbeeck J, Klunder HC, Mertens E, Halloran A, Muir G, Vantomme P (2013) Edible insects: future prospects for food and feed security. *Food and Agriculture Organization of the United Nations*.

Verhoeckx K, van Broekhoven S, Gaspari M, de Hartog-Jager S, de Jong G, Wichers H, van Hoffen E, Houben G, Knulst A (2013) House dust mite (Derp 10) and crustacean allergic patients may be at risk when consuming food containing mealworms proteins. *Clinical and Translational Allergy* **3 (Suppl. 3)**, 48.

Verkerk MC, Tramper J, van Trijp JCM, Martens DE (2007) Insect cells for human food. *Biotechnology Advances* **25**(2), 198-202.

Vijver M, Jager T, Posthuma L, Peijnenburg W (2003) Metal uptake from soils and soil-sediment mixtures by larvae of Tenebrio molitor (L.) (Coleoptera). *Ecotoxicol Environ Saf* **54**(3), 277-89.

Wilson ME, Lorente CA, Allen JE, Eberhard ML (2001) *Gongylonema* infection of the mouth in a resident of Cambridge, Massachusetts. *Clin Infect Dis* **32**(9), 1378-80.

Wisniewski HM, Sigurdarson S, Rubenstein R, Kascsak RJ, Carp RI (1996) Mites as vectors for scrapie. *Lancet* **347**(9008), 1114.

Yew KL, Kok VS (2012) Exotic food anaphylaxis and the broken heart: sago worm and takotsubo cardiomyopathy. *Med J Malaysia* **67**(5), 540-1.

Yi L, Lakemond CMM, Sagis LMC, Eisner-Schadler V, van Huis A, Boekel MAJSV (2013) Extraction and characterisation of protein fractions from five insect species. *Food Chemistry* **141**(4), 3341-3348.

Zagrobelny M, Bak S, Rasmussen AV, Jørgensen B, Naumann CM, Møller BL (2004) Cyanogenic glucosides and plant-insect interactions. *Phytochemistry* **65**(3), 293-306.

Zhou J, Han D (2006) Safety evaluation of protein of silkworm (*Antheraea pernyi*) pupae. *Food and Chemical Toxicology* **44**(7), 1123-1130.

Zhuang P, Zou H, Shu W (2009) Biotransfer of heavy metals along a soil-plant-insect-chicken food chain: field study. *J Environ Sci (China)* **21**(6), 849-53.

ANNEXES

<u>Annex 1</u>: Regulatory texts relating to insects

• <u>Concerning the rearing of insects:</u>

In France, there is currently no legislation applying to the rearing and marketing of edible insects. Nevertheless, there is one text concerning the rearing of non-domestic or wild species¹⁰. According to the French Environmental Code, the rearing of any non-domestic species of any kind for profit is subject to the requirement of a farming competency certificate and a prefectural authorisation to open a farming facility. As insects are non-domestic species, they fall within the scope of the "captive wildlife" regulations: operating a professional insect breeding facility therefore requires prior granting of a farming competency certificate and a prefectural authorisation to open, as mentioned in Articles L. 413-2 and 3 of the French Environmental Code. To access this dual authorisation regime, it is also necessary to prove a certain amount of experience and prior knowledge concerning the species in question, under the Ministerial Decree of 12 December 2000. It should be noted that there are no national animal protection measures specific to insects reared in captivity.

Concerning the hygiene rules applying to the production of animals for human consumption, these are the general rules established by the hygiene package, and more specifically by Regulation (EC) No 178/2002 aimed at food safety for humans. The hygiene package applies to all stages of production, processing and distribution of foodstuffs¹¹ and animal feed¹², and also implicitly includes insects.

Producers and distributors of insects and/or products derived from insects fall under the European regulations on the hygiene of foodstuffs (Regulation (EC) No 852/2004 (2004)), more precisely on those of animal origin (Regulation (EC) No 854/2004 (2004)) and on animal feed (Regulation ((EC) No 183/2005 (2005)). HACCP (hazard analysis and critical control points) must be applied.

With regard to the feeding of livestock intended for the production of food, the legislation stipulates that they can only be reared on authorised substrates. Indeed, in accordance with Article 3.6 of Regulation (EC) No 1069/2009, "any animal that is kept, fattened or bred by humans and used for the production of food" is a farmed animal (Regulation (EC) No 1069/2009 (2009)). In particular, these animals cannot be fed with raw materials prohibited in animal feed such as:

- slurry or solid manure (Annex III of Regulation (EC) No 767/2009),
- treated wood (Annex III of Regulation (EC) No 767/2009),
- kitchen and table waste (Article 11.1 (b) of Regulation (EC) No 1069/2009).

In contrast, waste from the production of bioethanol, such as wheat proteins and barley residues, is listed in the catalogue of raw materials (Regulation (EU) No 68/2013) and could therefore be used as a substrate for rearing insects.

¹⁰ <u>http://www.developpement-durable.gouv.fr/Les-etablissements-d-elevage-et.html</u>

Decree of 10 August 2004 laying down the general rules of operation for hobby farming facilities for non-domestic animal species.

¹¹ Foodstuff: any substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be ingested by humans.

¹² Animal feed: any substance or product, including additives, whether processed, partially processed or unprocessed, intended to be used for oral feeding to animals.

• <u>Concerning insects for animal feed</u>:

Regulation (EU) No 68/2013), cited above, also stipulates that insects can be a raw material for animal feed. It cites "whole or parts of terrestrial invertebrates [...] other than species pathogenic to humans or animals". The regulations on undesirable substances in animal feed (Directive 2002/32/EC (2002)) do not exclude insects which, as a raw material, are therefore subject to its requirements.

The regulations on animal by-products and derived products (Regulation (EC) No 1069/2009 (2009)) also apply, including for products imported into Europe. According to this latter text, invertebrates, and therefore insects, that are non-pathogenic to humans are regarded as Category 3 material and can be used:

- for feeding livestock animals if they have been transformed into hydrolysed proteins via a validated chemical hydrolysis process;
- in petfood under certain processing conditions.

They may be used as such for fur animals, zoo animals, circus animals and other captive wild animals (after first obtaining a national derogation and after specific authorisation of end users, the producer is also required to be declared).

Regulation (EC) No 999/2001 (2001) prohibits the use of processed animal proteins (PAP) for livestock animals (processed animal proteins are derived exclusively from by-products of animals fit for human consumption (Category 3)). Since 1 June 2013, PAPs from non-ruminants have been reintroduced in food for aquaculture species (Regulation (EC) No 56/2013 (2013)). PAPs are produced from by-products collected in slaughterhouses and processed in by-product facilities, which excludes insects. If the legislation were to evolve, the use of PAPs derived from insects would be limited to non-ruminant animals (pigs, poultry, fish), and the ban for ruminants would remain in place.

<u>Concerning insects for human food:</u>

Regulation (EC) No 258/97 (1997) concerning novel foods and novel food ingredients stipulates that foods which have not been used for human consumption to a significant degree in the European Union before 15 May 1997 must undergo a risk assessment prior to being marketed. Currently, this regulation is ambiguous as to the interpretation of "significant degree" of consumption. In addition, it is imprecise in its current wording because its scope only covers parts of animals ("food ingredients isolated from animals"), and not whole insects.

At the present time, the bodies of the Member States responsible for assessing an application for pre-market authorisation are the first to be involved. For France, this is the DGCCRF. When the DGCCRF deems that a food falls within the scope of Regulation (EC) No 258/97, it issues an opinion, based on the expert appraisal of ANSES (favourable or unfavourable, with or without reservations and conditions), in the form of an initial assessment report, which is sent to the European Commission. The Commission then distributes this initial assessment report to all the Member States for comments and objections. If no reasoned safety objections are submitted, the opinion of the DGCCRF is endorsed and the novel food may or may not be placed on the market. If reasoned safety objections, or any other objections to the conclusions of the opinion, are presented, a Commission on marketing authorisation is required, which implies in most cases an additional assessment carried out by the European Food Safety Authority (EFSA). The

novel foods whose placing on the European market have been authorised or refused are advertised to the public by the EU. Once authorised, a novel food can be proposed in any Member State.

To date, no admissible marketing authorisation application has been submitted in Europe for insects or products derived from insects intended for human food. Therefore, no insect or insect derivative can be placed on the market for human food.

The purpose of the draft of the new regulation on novel foods (COM (2007) 872 (2007)) is to streamline the authorisation procedure, and improve its effectiveness and transparency. It introduces a method of assessing safety that is both faster and better suited to traditional foods from third countries whose safety in past use has been demonstrated. Under this regulation, it is probable that insects will be attached to this category, which encompasses food that has been a component of the normal diet for at least a generation in a large proportion of the population of the third country. If the applicant is able to provide documented data to demonstrate that the novel food has a completely safe history of use in a third country, it may be authorised to be placed on the market. Where appropriate, the applicant will have to conduct a risk assessment.

		Antinutritional factors			
Insect species (or order)		Phytic acid	Total oxalate	Hydrocyanic acid	Tannin
Gymnogryllus lucens	(Orthoptera – Gryllidae)	0.28	13.20	2.19	0.33
Heteroligus meles	(Coleoptera – Dynastidae)	0.28	29.00	2.73	0.38
Rhynchophorus phoenicis	(Coleoptera – Curculionidae)	0.29	19.32	2.53	0.48
Zonocerus variegatus	(Orthoptera – Pyrgomorphidae)	0.28	26.40	3.20	0.43
Cirina forda	(Lepidoptera – Saturniidae)	1.02	4.11	Not determined	Not detected
Anaphe venata	(Lepidoptera – Notodontidae)	0.19	Not determined	Not determined	0.07
Ant		0.20	Not determined	Not determined	0.04
Termite		0.25	Not determined	Not determined	0.09
Winged termite		0.11	Not determined	Not determined	0.02
Katydid		0.11	Not determined	Not determined	0.10
Cricket		0.32	Not determined	Not determined	0.90
Cochineal		0.23	Not determined	Not determined	0.11
Tree hopper		Not determined	Not determined	Not determined	0.10

Annex 2 : Antinutritional factors found in several insect species (mg/100g of dry weight) (Belluco, Losasso et al. 2013)

Sources: (Adeduntan 2005; Adesina 2012; Ekop, Udoh et al. 2010; Omotoso 2006)