

Toxicological aspects of human consumption of insects or insect proteins in Europe

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- Toxicological hazards
 - Natural toxins
 - Contaminants
- Risk assessment of edible insects and proteins
 - Novel food regulation requirement
 - A challenge for toxicologists
 - Exemples
- Conclusion



Potential hazard induced by edible insect consumption



- Chemical



- Natural toxins: Phanerotoxic and cryptotoxic insects
 - Contaminants: mycotoxins, pesticides, heavy metals, ..
- ## - Microbiological (C Nielsen-Leroux)
- ## - Allergy (R Gadisseur)



Natural toxins

Phanerotoxic:

- ❖ Organs for the synthesis of poisons, as in the case of bees and ants.
- ❖ Toxins are generally inactivated in the digestive tract.
- ❖ Potential risks during the oral and esophageal passage of stings.
- ❖ Some biogenic amines can become dangerous if present at a high level

Cryptotoxic

- ❖ Toxins as a consequence of synthesis or accumulation
- ❖ Localized in specific structures or diffused in different body areas.
- ❖ Potentially dangerous for humans after ingestion

Belluco et al, 2013



Natural toxins

Defensive secretions that may be reactive, irritating, toxic

Several chemical families:

- carboxylic acids, alcohols, aldehydes,
- alkaloids, ketones, esters,
- lactones, phenols, 1,4-quinones, Hydrocarbons
- Steroids

Van der Spiegel et al, 2013



Natural toxins

Tenebrio molitor :



- Quinones,
- Genotoxic and carcinogenic
- Only mature adult not larvae



Beetles, *Dytiscidae* family

- Metabolic steroids (including testosterone and dihydrotestosterone)
- Growth retardation, hypofertility, masculinization in females, oedema, jaundice, liver cancer

« *Tenebrio molitor* larvae »
Rum, Tirol, Austria.
« *Tenebrio molitor* MHNT »
D. DescouenTravail
personnel.

NVWA, 2012, Belluco et al, 2013





Natural toxins

Coleoptera and Lepidoptera

- Cyanogenetic substances
- Inhibition of enzymes such as succinate dehydrogenase and carbonic anhydrase,
- Inhibition some metabolic pathways like oxidative phosphorylation (affinity for ferrocytochrome oxidase).

Encosternum (= Natalicola) delegorguei

- Zimbabwe, South Africa
- Caustic fluid
- Severe pain in eyes, blindness

Blum 1994, Scholtz, 1984





Natural toxins



Lytta vesicatoria:

- Cantharidine
- Eggs, ovaries
- Aphrodisiac properties
- Bladder and urethra irritation, lethal

70 Dictionnaire de Médecine Pratique

deux formules de préparations cantharidiennes. Nous les empruntons au docteur Langlebert :

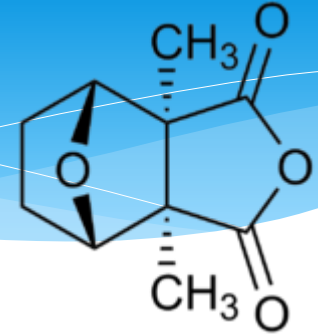
1° Eau de menthe	60 grammes.
Sirop simple	30 —
Teinture de vanille	4 —
Teinture de cantharides	4 à 20 gouttes.

A prendre par cuillerée à dessert, chaque cuillerée à une heure ou une demi-heure d'intervalle.

2° Eau de menthe	60 grammes.
Teinture de cannelle	4 —
Sirop simple	30 —
Teinture de noix vomique	4 à 10 gouttes.
— de cantharides	

A prendre comme la précédente.

L'action irritante et locale des cantharides sur la muqueuse urinaire ne permet pas de l'employer d'une manière suivie. Elle peut conduire au priapisme et au satyriasis.

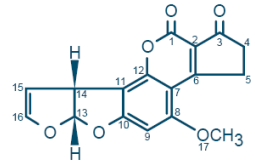


Lepidoptera ygaena, Italy

- potentially dangerous
- cyanogenetic glycosides.
- increasing risk among undernourished subjects with a low sulfur diet.



Contaminant-Mycotoxins



Aflatoxin B₁

Mycotoxins are

- Secondary metabolites produced by fungi
- Thermostable,
- Several families:
 - Zearalenone: oestrogenic effect
 - Fumonisin: oesophagus cancer ?
 - Ochratoxins (OTA): neurotoxic, immunotoxic, carcinogenic
 - T-2, HT-2 toxins: haematotoxic, immunotoxic
 - DON: anorexia, growth retardation
 - Aflatoxins: genotoxic, carcinogenic (liver)

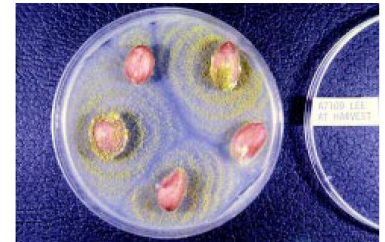


Figure 4.1. Growth of *Aspergillus flavus* (yellow-green fungus) from two of five surface sterilized peanuts placed on a nutrient culture medium. Photograph courtesy of R. J. Cole, USDA, ARS, National Peanut Research Laboratory, Dawson, Georgia.

Consumption of mycotoxin-contaminated edible insects may pose a risk for Humans



Contaminant-Mycotoxins

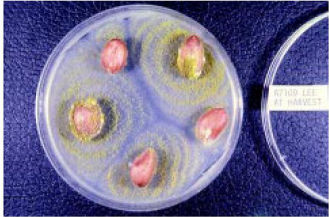
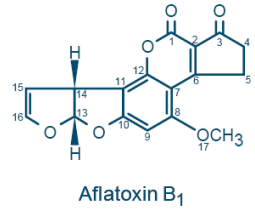


Figure 4.1. Growth of *Aspergillus flavus* (yellow-green fungus) from two of five surface sterilized peanuts placed on a nutrient culture medium. Photograph courtesy of R. J. Cole, USDA, ARS, National Peanut Research Laboratory, Dawson, Georgia.

Schrögel et al, 2019

Zearalenone, OTA, T-2 toxin: *Alphitobius diaperinus*, *Tenebrio molitor*, *Zophobas atratus* (2014)

DON(s): *Tenebrio molitor* (2017, 2017, 2019)

Zearalenone(s), DON(s), Aflatoxins, OTA: *Hermetia illucens* (2017)

Aflatoxins (B₁, M₁): *Tenebrio molitor*, *Hermetia illucens* (2017)

Zearalenone(s), DON(s), Aflatoxins: *Alphitobius diaperinus*, *Hermetia illucens* (2018)

Zearalenone: *Tenebrio molitor* (2019)



Contaminant-Heavy metals



Rhynchophocus phoenicis and *Anapleptes trifaciata*

- 0,03 mg lead /kg insects et 0,06 mg lead /kg insects (Banjo et al., 2010).

Larvae of *Rhynchophorus phoenis* crudes, harveted in Nigeria

- 2,1 mg lead/kg insects (Ekpo, 2010).

Antheraea perni, silkworm pupa, China

- 1,13 mg lead /kg d'insects (Zhou et al., 2006)

Larvae *Tenebrio molitor*

- Cadmium and lead (Vijver et al., 2003).





Contaminant - Heavy metals

Poma et al. (2017)

Table 4

Measured levels of metals in edible insects and insect-based food (mg/kg ww).

species	Sample	Cr	Co	Ni	Cu	Zn	As	Cd	Sn	Pb
<i>Edible insects</i>										
<i>Galleria mellonella</i>	EI-1	0.17	<0.03	0.04	3.47	25.80	<0.03	0.04	<0.03	<0.03
<i>Locusta migratoria</i>	EI-2	0.12	<0.03	0.20	9.12	37.10	<0.03	0.03	<0.03	<0.03
<i>Tenebrio molitor</i>	EI-3	0.18	0.05	0.28	5.81	58.60	<0.03	0.06	<0.03	<0.03
<i>Alphitobius diaperinus</i>	EI-4	0.15	<0.03	<0.03	7.72	54.10	<0.03	<0.03	<0.03	<0.03
<i>Locusta migratoria</i>	EI-5	0.11	<0.03	0.20	5.31	38.30	<0.03	<0.03	<0.03	<0.03
<i>Insect-based food</i>										
<i>Locusta migratoria</i>	EI-6	0.12	<0.03	<0.03	1.62	6.44	<0.03	<0.03	<0.03	<0.03
<i>Alphitobius diaperinus</i>	EI-7	0.12	<0.03	0.18	1.67	11.40	<0.03	<0.03	<0.03	<0.03
<i>Acheta domesticus</i>	EI-8	0.24	<0.03	0.18	0.85	8.47	<0.03	<0.03	<0.03	<0.03
<i>Alphitobius diaperinus</i>	EI-9	0.12	<0.03	0.23	1.51	7.33	<0.03	<0.03	<0.03	<0.03

Levels detected in insect foodstuffs are compliant with Regulation (EU) 1881/2006 for contaminants.



Contaminant - Heavy metal

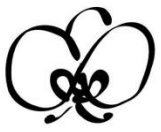
Table 3: Concentrations of cadmium, lead, mercury and arsenic (mg/kg dry weight) in farmed insects (MD1 to MD5: house fly (*M. domestica*); CV1 and CV2: blue bottle (*C. vomitoria*); CH: blow fly (*Chrysomya spp.*); HI: black soldier fly (*H. illucens*) reared using a variety of production methods at different geographical locations.

Element	Feed material max. limit ^(a)	Complete feed max. limit ^(a)	MD1	MD2	MD3	MD4	MD5	CV1	CV2	CH	HI
Cadmium ^(b)	2	0.5	0.334	0.625	0.348	0.711	0.723	0.02	0.018	0.370	0.120
Lead ^(b)	10	5	0.46	1.16	0.249	0.058	0.333	<0.001	<0.001	<0.001	<0.001
Mercury ^(c)	0.1	0.1	0.004	0.035	0.038	0.002	0.004	<0.002	<0.002	0.008	0.007
Arsenic ^(b)	2	2	0.191	0.408	0.094	0.161	0.079	0.009	0.004	0.734	0.142

(a): EU maximum limit in feed material and complete feed for farm animals based on 88% dry matter.

(b): Commission Regulation (EU) No 1275/2013 of 6 December 2013 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels for arsenic, cadmium, lead, nitrites, volatile mustard oil and harmful botanical impurities.

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



Contaminants- Pesticides



Migratory locust, Kuwait (Saeed et al, 1993)

- Lindane 2.2 $\mu\text{g}/\text{kg}$ I, Aldrin 6.2 $\mu\text{g}/\text{kg}$, Sumithion 740 $\mu\text{g}/\text{kg}$ I, Malathion 49.2 $\mu\text{g}/\text{kg}$ I

Poma et al, 2017

- Untargeted screening analysis of pesticides
- vinyltoluene,
- tributylphosphate (75% of the samples),
- pirimiphos-methyl (identified in 50% of the samples)
- Others pesticides < LOQ

All pesticides used against insects are potentially dangerous for consumers, particularly if the products have been obtained by wild harvesting rather than controlled farming.



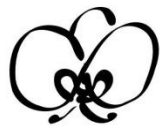
Efsa (2015)

The possible presence of biological and chemical hazards in food products derived from insects depends

- * on the method of production,
- * the substrate (feed) of insects,
- * the insect species,
- * the stage of lifecycle at harvesting,
- * the method of further processing.



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Novel Foods Regulation (EU) 2283/2015

January 2018



Insects and insect-derived products

- Are considered to be novel foods
- Are subject to the novel foods approval procedure by Efsa

SCIENTIFIC OPINION



ADOPTED: 21 September 2016

doi: [10.2903/fj.efsa.2016.4594](https://doi.org/10.2903/fj.efsa.2016.4594)

Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283



Novel Foods Regulation (EU) 2283/2015

January 2018



Novel Food guidance :

2.10. Toxicological information

- Tiered toxicity testing approach
- For food additives (EFSA ANS Panel, 2012)

2.10.7.1. Insects

- Isolated from, or are produced from, farmed insects,
- Taking into account the species and substrate
- Methods for farming and processing.

Insects collected from the wild may bear additional biological and chemical hazards which should be considered and addressed.



Novel Foods Regulation (EU) 2283/2015

TIER 1

- **Absorption**
- **Genotoxicity**
 - In vitro testing
- **Toxicity**
 - Extended 90-day toxicity study



Triggers for considering Tier 2

- *Systemic availability*
- *Toxicity in the 90-day study*
- *Genotoxicity in vitro*

TIER 2

- **ADME**
 - Single dose
- **Genotoxicity**
 - In vivo testing
- **Toxicity (stand-alone or combined)**
 - Chronic toxicity
 - Carcinogenicity
- **Reproductive & Developmental toxicity**
 - EOGRTS
 - Prenatal developmental toxicity



Triggers for considering Tier 3

- *Bioaccumulation*
- *Positive in vivo genotoxicity*
- *Chronic toxicity/carcinogenicity*
- *Reproductive & Developmental toxicity*

TIER 3

- **ADME**
 - Repeated dose, volunteer studies
- **Carcinogenicity**
 - Mode of action
- **Reproductive & Developmental toxicity**
- **Specialised studies**
 - e.g. immunotoxicity, neurotoxicity, endocrine activity, mode of action



Tier 1

Toxicokinetic:

- * Systemic exposure/systemic availability
- * Absorption, distribution, metabolism
- * Not required for insect, to be justified



Genotoxicity:

- * Detection mutagen/clastogen effects
- * On somatic cells
- * Involvement in neoplastic transformation, cancer



Genotoxicity

- No single validated test method can provide information on all the genetic endpoints.
- *In vitro* genotoxicity tests (Efsa Guidance):
 - Bacterial reverse mutation assay Ames test
 - *In vitro* mammalian cell micronucleus test
- *In vivo* genotoxicity testing
 - *In vivo* micronucleus test
 - *In vivo* comet assay
 - Transgenic rodent mutagenicity assay



Subchronic toxicity studies

OECD 408

Objective:

- Determine the toxicological profile of the substance
- Repeated administration to experimental animals
- Over a prolonged period of time (1/10 lifetime, 90 days for murine, 1 year for dog).

Data:

- the target organs
- the nature and severity of any effects
- the dose response relationships
- Dose without any toxic effect





Subchronic toxicity studies



According to the relevant OECD guidelines 408, the studies should include:

- measurements of food consumption, body weight
- hematological examinations
- clinical biochemical determinations in plasma and serum, urinalysis
- gross necropsy, determination of organ weight
- histopathology



Insect toxicity testing

A challenge for toxicologists



Whole insects can be eaten

- raw, dried, crushed, textured, pulverized, or ground,
- heated such as cooked, boiled, fried, roasted, toasted, extruded, and canned
- or preserved by freeze-drying or cryovacking after degutting or fasting.

Food processing can also introduce toxic substances by chemical reactions of

- substrates of insects
- and other ingredients, such as heterocyclic aromatic amines, acrylamide, chloropropanols, and furans



Insect toxicity testing

A challenge for toxicologists



Toxicological studies	Protein	Insect
Genotoxicity tests		
Ames test	False positive	Whole, Crude, cooked, Lyophilised, Powder ?
Micronoyau in vitro/in vivo	OK	Whole, Crude, cooked, Lyophilised, Powder ?
Subchronic toxicity (90 days, rat)	Nutritionnale unbalance (Enzymes ou Pt GMO)	Whole, Crude, cooked, Lyophilised, Powder ?



Molecule (Insect Protein ?)

Insects



- * Toxicokinetic
- * Subchronic toxicity
- * Genotoxicity <0
- * Chronic/cancerogenicity
- * Reproduction and developmental toxicity



No Observed
Adverse Effect Level
(NOAEL)
mg/kg body weight/day



$$ADI = NOAEL/100$$



ADI > Exposure
No risk for the consumer

- * 0 Toxicokinetic
- * Subchronic toxicity
- * Genotoxicity <0
- * Toxic effects, highest dose ?



No Observed
Adverse Effect Level
(NOAEL)
mg/kg body weight/day

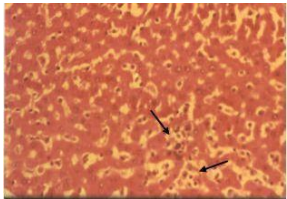


No ADI
Risk characterisation ?
Margin of Safety ?
Cancerogenicity ?
Reproduction ?





Edible Larvae of *Cirina Forda* (Westwood) Crude or cooked ?



- Vehicule : distilled water,
- 3 doses 1750, 2250 et 3700 mg/kg w/dy
- Extract of crude or cooked larvae
- 14 days, rats
- Extracts of crude larvae: Toxic effects on liver, kidneys, lungs
- Extracts of cooked larvae: Toxic effects on lungs only

Akinnawo et al, 2005



Protein of silkworm (*Antheraea pernyi*) pupae

- Genotoxicity assays
 - Ames: negative
 - Micronucleus *in vivo* (mice): negative
 - mouse sperm abnormality test: negative
- Acute toxicity:
Maximum tolerate Dose 15 g/kg bw, mice
- Subacute toxicity study: 30 days
 - Rats, males et females, 5/dose
 - Doses tested : 0, 300, 750, 1500 mg/kg bw/day
 - Hematological, clinical parameters, urinalysis, food consumption, weight, histopathology



No adverse effect



Genotoxicity assays



Genotoxicity:

Human lymphocytes
chromosome aberration: negative
micronucleus tests: negative

Zonocerus variegatus (Orthoptera: Pyrgomorphidae)

Oryctes boas (Solanales: Solanaceae), *Saga ephippigera*
ephippigera et *Callimenus dilatatus*, dung beetles (*Onitis* sp.),

grasshopper (*Caelifera* sp.) and mole crickets (*Gryllotalpa* sp)

Migratory locust (*Locusta migratoria* L.)

Türkez et al, 2010, 2014, Menis et al, 2012, Koc et al, 2014.,



Toxicological studies

Test item preparation



Protaetia brevitarsis larvae

Powder suspended in distilled water

Tenebrio molitor larvae

Freeze-dried Powder

Cricket, *Gryllus bimaculatus*

Dehydrated, freeze-dried at -70°C

Crude ? Cooked ?

Noh et al, 2018, Han et al, 2014, 2016, Ruy et al, 2016



Toxicological studies



Protaetia brevitarsis larva

Ames test and in vivo micronucleus: negative
90-day study study: No adverse effect,
NOAEL= 3000 mg/kg bw/day

Tenebrio molitor larvae

Ames test and in vivo micronucleus: negative
28-day study/ 90-Day study: No adverse effect,
NOAEL= 3000 mg/kg bw/day

Cricket, *Gryllus bimaculatus*

90-day study study: No adverse effect,
NOAEL= 5000 mg/kg bw/day

Noh et al, 2018, Han et al, 2014, 2016, Ruy et al, 2016



CONCLUSION



Potential chemical hazard induced
by edible insect consumption

- Natural toxins ? Risk assessment procedure
- Contaminants: mycotoxins, pesticides, heavy metals ? Could be solve if rearing of insect is optimised by using a substrate of constant composition and specified quality.





CONCLUSION

Efsa, Nov 2019 :

No opinion has been published ...Why ?

The clock is stopped, Why ?



Gao et al, 2018:

Comprehensive review

Toxicological assessment of edible insects in China

Safe, great potential as novel food resources.

Need a more complete toxicological assessment according to the new standard so that they can become more favorable internationally.



Thank you for your attention

