Mushrooms traded as food. Vol II sec. 2

Nordic Risk assessments and background on edible mushrooms, suitable for commercial marketing and background lists. For industry, trade and food inspection. Risk assessments of mushrooms on the four guidance lists

OYSTER (PLEUROTUS OSTREATUS)

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Jørn Gry and Christer Andersson

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Introduction to Volume 2, section 2

The project *Mushrooms Traded as Food* aims at improving the in-house control and thereby the safety of traded food mushrooms in the Nordic countries by basing the risk management on scientific risk assessments.¹

Volume I, containing the questionnaire and the guidance lists, is available in five versions: in Danish, Icelandic, Norwegian, Swedish and English (see www.norden.org).

Volume II, section 1 and *Volume II, section 2* of “Mushrooms Traded as Food” are in English and contain the background information and risk assessments of food mushrooms to be used in the in-house control by trade and industry and the public food inspection.

The two background reports compile knowledge on which the guidance in Volume I is based. The project has established guidance lists 1 to 4 on mushrooms that are suitable or not suitable to be traded as food. In order to be transparent and facilitate the decision on which mushrooms are suitable on the various lists produced within the project, a risk assessment was performed for each mushroom species in the four lists. These risk assessments were performed according to the principles laid down in the Codex Alimentarius Risk Analysis strategy. The mushroom species considered to be included on any of the lists were determined by earlier activities within the National Authorities responsible for food safety in the Nordic countries as well as information from the Nordic mycological societies.

Volume II, section 1 gives some details on mushrooms, contaminants, intoxications and relevant legislation, and presents the guiding tools for business operators and food control, not least the Nordic guidance lists on mushrooms. These guidance lists have been compiled to be used together with the questionnaire in Volume I of the project report.

¹ The project is reported in two volumes, one is a questionnaire with guidance lists giving the direct advices (Volume I), the other supplying supporting background information (Volume II).

Volume II, section 2 constitutes risk assessments of all the about 110 mushrooms in the four guidance lists. Each risk assessment contains information on the preferred scientific name, the preferred name in English and in each Nordic language, a picture of the mushroom, the chemical structure of identified natural toxicants and certain other bioactive constituents in the mushroom species and not least risk assessments of the mushrooms in the lists with recommendations and the references used for the assessments. The references comprise the scientific publications available based on searches in the databases SciFinder and possibly also PubMed up to the dates in 2012 indicated in the monographs on the 110 mushrooms and their known toxic constituents.

The project working group² agreed on the structure of Volume II, section 2.

The risk assessments were performed by Jørn Gry and Christer Andersson.

For acknowledgements see *Volume II, section 1*. For this Volume II, section 2, special thanks are given to Folmer D. Eriksen, Henning Knudsen and Thomas Læssøe for their many contributions.

² Bente Fabech (chairperson) and Lulu Krüger, Danish Veterinary and Food Administration, Christer Andersson, National Food Agency, Sweden, Jørn Gry (consultant), Denmark, Birgitte Lyrån and Laila Jensvoll, Norwegian Food Safety Authority, Niina Matilainen and Annika Nurttila, Finnish Food Safety Authority Evira, Finland and Grímur Olafsson, Public Health Authority of Hafnarfjörður and Kópavogur, Iceland.

1. Risk analysis and the steps of risk assessment

Since the middle of the 1990s food safety issues have been more formalized dealt with by the principle of risk analysis developed by Codex Alimentarius. When a hazard has been identified, which most often is done by scientists like food toxicologists, food microbiologists, food inspectors, medical doctors or other professionals, risk managers request a risk assessment to be performed. However, also consumers, politicians and others do initiate the process of risk analysis, when they observe potential hazards.

According to the risk analysis principle it is the task of scientists to perform the risk assessment. The risk assessment will, together with other legitimate factors, be used by risk managers in deciding on how to manage the identified risk. The other legitimate factors can be e.g., traditional use of a process like the smoking process and use of traditional foods like False Morel (*Gyromitra esculenta*). Even though smoking may cause contamination with carcinogenic aromatic hydrocarbons (PAH) in the smoked food, and False Morel contains inherent, suspected carcinogenic components, the smoked foods and the mushroom can be a traditional part of a national diet and therefore, the risk be accepted in the population. During the whole risk analysis process risk assessors and risk managers are entitled to communicate with communication experts and interested parties in a process called risk communication. This division of the tasks was created to increase the transparency in the decision making process and allow a wider audience to participate.

Risk assessments of mushrooms in the Nordic countries have to be performed within the legal framework of the European Community for food-stuffs. This is the case also for Norway and Iceland having co-operation agreements with the EU. However, there is no harmonized EU legislation specifically related to mushrooms. On the other hand, there are some specific legislation that might have a bearing on which mushrooms can be marketed, like the regulation on Novel Food, see Vol. II, section 1.

Overall, the risk analysis comprises three interrelated areas:

- Risk assessment (the scientific part).
- Risk management (the administrative part).
- Risk communication (throughout the risk analysis process).

The scientific risk assessment can be described as a process developing over four phases: hazard identification, hazard characterization, exposure assessment and risk characterization.

Hazard identification is “the identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of food.”

Hazard characterization: When a hazard has been identified in a risk assessment, hazard characterization follows. Codex Alimentarius defines hazard characterization as “the qualitative and/or quantitative evaluation of the nature of the adverse health effect associated with biological hazards, chemical and physical agents, which may be present in food. For chemical agents, a dose-response assessment should be performed. For biological or physical agents, a dose-response assessment should be performed if data are obtainable.” Scientists often make hazard characterization based on animal testing. Included in the hazard characterization should be a description of uncertainties.

Exposure assessment: The third phase of the risk assessment is exposure assessment, which by Codex Alimentarius is defined as “the qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant.” Exposure assessment is a subject dealt with in many international guidelines on for example food additives, pesticides, flavourings etc. Exposure assessment is based on scientific knowledge on consumption patterns, surveys etc. Since consumption surveys are very expensive and as the consumption pattern changes over time, the assessments are in many cases based on models simulating the real intake of food. These models are often developed through international cooperation and accepted in the guidelines for risk assessment.

Exposure assessments of the bioactive compounds in mushrooms are in most cases difficult to perform. This is not only linked to a generally poor understanding of the quantities of various bioactive compounds in mushrooms, but also to a very limited knowledge on the average consumers’ consumption of various types of mushrooms, or to the actual consumption (acute or long-term) by those consuming mushrooms or to sensitive consumer groups (e.g. children and elderly). The poor knowledge on mushroom consumption was illustrated in 2008 when a German Food Control Authority noted that nearly all samples of dried

Cep (*Boletus edulis*) on the market contained nicotine above statutory pesticide residue levels. Reacting on the new information, the European Commission requested the European Food Safety Authority (EFSA) to estimate the mushroom intake in Europe by using food consumption data on raw cultivated and wild mushrooms provided by Member States for the development of the EFSA Pesticide Residue Intake Model. In order to respond to the request, EFSA asked Member States to deliver recent and detailed food consumption data for adults and children concerning different types of mushrooms, including wild mushrooms such as Cep (*Boletus edulis*). Only a few Member States were able to deliver such data. Frequently, these food descriptors were not available in the national databases. Mushroom consumption in adults was obtained from Ireland, Italy, Finland and France. The same type of data for children were available from the Netherlands, Ireland, Belgium, Italy and France. Most of these countries reported consumption figures for “total mushrooms”, only Italy and Finland presented figures for the consumption of Cep. For most of the known, traded food mushroom species there will be no intake data available.

Risk characterization is the last step in the risk assessment process. Risk characterization is defined as “the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment.”

From the statements above it is clear that one of the important issues for the scientists involved in the risk assessment is to point out the *uncertainties* and assumptions made during the assessment, when reporting the risk assessment results. A general discussion on uncertainties in risk assessment is found in the project reports Food Safety in Europe: Risk Assessment of Chemicals in Food and Diet (2002) and Risk Characterisation of Chemicals in Food and Diet (2003). The uncertainties in the risk assessment, including some of the fundamental assumptions, are important for the management decisions and should be incorporated in the risk assessment as uncertainties.

2. Introduction to mushroom risk assessments

Lists of mushrooms

As a basis for differentiating between mushrooms suitable to be marketed as food mushrooms and those that are not, risk assessments were performed on all mushroom species suggested by a Nordic country to be considered as a food mushroom, when the project started in 2008.

The preliminary list of mushrooms, suggested to constitute the mushrooms suitable as traded food in the Nordic countries, was produced from available national lists of food mushrooms and after consultation of Nordic mycological societies. It was also considered that the lists might not be exhaustive and that the lists established in the project would need to be reviewed, if new edible mushrooms should be included and to be in line with most recent science.

To determine whether the mushroom species present on the joint Nordic list were suitable to be kept on the list a risk assessment monograph was prepared for each species and toxic look-alike mushrooms were identified.

The resulting 110 mushroom monographs are presented in alphabetical order according to scientific name of the mushroom in the next chapter.

Based on the outcome of the risk assessments and whether there are toxic look-alikes, four new lists were established from the joint Nordic list compiled for this project. These lists are included in Volume I and in Volume II, section 1 of the reports.

The lists are:

- List 1 Edible mushrooms suitable for commercial marketing (cultivated and/or wild).
- List 2 Wild edible mushrooms, where the identity has to be documented by recognized experts, to be suitable for commercial marketing.
- List 3 Wild edible mushrooms, which may easily be mistaken for poisonous look-alikes and therefore are not regarded as suitable for commercial marketing.

- List 4 Wild mushrooms earlier regarded as edible, but which are suspected to cause acute or long-time adverse effects after ingestion and therefore not regarded as suitable for commercial marketing.

Additionally, a list of toxic look-alikes referred to in the risk assessments on mushrooms in list 3, has been made, see Chapter 4.

Literature used

The risk assessments are based on information in the scientific literature. The scientific literature was identified in database searches in SciFinder and when appropriate in PubMed during 2012 as indicated for each of the mushrooms assessed, and in recent reference books on mushrooms (generally, published in 2000 or later) written by recognized mycologists.³ References to these publications are given in the assessments. Other documents used when preparing these assessments included for example Codex Alimentarius standards (FAO/WHO) on mushrooms, applicable risk assessments performed by EFSA (the European Food Safety Authority) panels, and regional/national legislation/recommendation regarding mushrooms/fungi.

Photos and structural formulae

Photos showing the typical appearance of all the mushrooms assessed are included in the 110 individual risk assessments, as well as structural formulae of bioactive constituents,⁴ especially toxicants in the mushroom.

Concerning photos, it *has to be stressed*, that one picture of a mushroom species will never cover all variations in the appearance of the mushroom in nature. The natural appearance can differ significantly.

The structural formulae have been worked out using ChemDraw Ultra Version 8.0.

³ A list of such handbooks is included in Vol. II, section 1.

⁴ Bioactive substances in mushrooms are inherent non-nutritive constituents with potential health protective and/or potential toxic effects when ingested (Gry *et al.*, 2007).

Toxic look-alikes

The toxic look-alikes referred to in the risk assessments of mushrooms in list 3 are not risk assessed, but there are scientific and vernacular names, a very short statement and a photo of each of them included in Chapter 4.

Nomenclature

All four lists give preferred scientific names as well as some commonly used synonyms. In addition to the scientific names, the preferred names in the English and Nordic languages are given. Other vernacular names used are also included, as are some of the names used in trade. The number of synonyms and trade names is not exhaustive.

If no preferred names were available from the primary sources below, the names are shown in brackets. Also synonyms are given in brackets. Trade names are additionally provided with hyphens.

General labelling regulations have to be followed regarding marketed mushrooms. In relation to this, it is recommended to use the scientific names and/or vernacular names given in the four lists.

The nomenclature used for the mushrooms in the risk assessment and in the guidance lists 1–4 is the following:

Scientific names

Preferred scientific names are taken from Index Fungorum/Species Fungorum (2012) as far as the “Current Name” is given (by June 2012). The names in Index Fungorum/Species Fungorum and the names in “Funga Nordica”, Knudsen & Vesterholt (2012) are in most cases identical. Derivations are indicated in the risk assessment. If not available in these extensive compilations, a case by case decision was taken after consulting the Nordic mycological societies.

Common scientific synonyms are generally selected from Index Fungorum/Species Fungorum (2012) and Knudsen & Vesterholt (2012).

English names

Preferred English names are taken from British Mycological Society (2012). If an English name is not available from this database, English names, as well as synonyms and trade names are taken from other sources (indicated within parenthesis).

Danish names

Preferred Danish names are taken from the database of the Danish Mycological Society (2012). Danish synonyms and trade names are taken from other sources.

Finnish names

Preferred Finnish names are generally taken from the publication: *Suomen helttasienten ja tattien ekologia, levinneisyys ja uhanalaisuus* (2005) by the Finnish Environment Institute SYKE (Suomen ympäristökeskus).

Icelandic names

Preferred Icelandic names were taken from Sveppabókin by the Icelandic mycologist Helgi Hallgrímsson. (2010). If Icelandic names were lacking, such names have in several cases been allocated to the mushrooms by consultation with Gudridur Gyda Eyjolfsdóttir (2011), Icelandic Institute of Natural History.

Norwegian names

Preferred Norwegian names are taken from the database of the Norwegian names for fungi, prepared by the Norwegian Mushrooms Name Committee (2011).

Swedish names

Preferred Swedish names have been provided by ArtDatabanken (2011). Names of Ascomycetes are taken from Eriksson (2009). The names of some mushrooms not considered by the above sources are given names according to Aldén and Ryman (2009).

Studies and constituents assessed

Studies on whole mushrooms, extracts and fractions thereof, without any characterization of composition, especially of bioactive constituents, are generally not included in the risk assessments. The results from such studies are very difficult to reproduce, as the composition, especially of the bioactive constituents, may vary considerably.

Mono-, di- and polysaccharides, proteins and individual amino acids and lipids in mushrooms are generally not included in the assessments. However, certain proteins (e.g., lectins) and amino acids (e.g., pleurocybellaziridine) are included, as they are suspected to cause adverse effects. Pigments are only included, if they have been suspected to be

biologically active and especially to give rise to adverse effects, e.g., in Brown Rollrim (*Paxillus involutus*) and Ugly Milkcap (*Lactarius necator*).

Habitat and occurrence

The habitat, way of growing (saprotrophic, parasitic or ectomycorrhizal), frequency and distribution (generally in the Nordic countries) are for Basidiomycetes, taken from Knudsen & Vesterholt (2012), for other mushrooms from Hansen & Knudsen (1992; 1997; 2000) or in a few cases from other sources. Exceptions are generally given by footnote for the individual mushroom.

Edibility

Edible mushrooms or fungi are either wild mushrooms or mushrooms that have been cultivated, and which are suitable for use as a food after appropriate processing. Not all edible mushrooms are suitable as mushrooms traded as food.

The edibility is for most of the 110 mushrooms designated “edible.” The edibility is further elaborated for “culinary mushrooms” like Cep (*Boletus edulis*), Chanterelle (*Cantharellus cibarius*) Morel (*Morchella esculenta*), and certain truffles (*Tuber magnatum* and *T. melanosporum*).

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3. Mushroom risk assessments

Risk assessment of the mushrooms in the guidance lists 1–4

The risk assessments in this chapter are listed in alphabetical order according to the scientific names of the mushrooms. The preferred vernacular names in the five Nordic languages and English are given in each assessment. If a name is only known in, e.g. Norwegian, then use the “search function” when searching in the electronic report to find the relevant risk assessment.

The nomenclature used is explained in the previous Chapter 2. Synonyms to preferred names are given in brackets and trade names are in addition provided with hyphens. Remarks to the nomenclature used are given as footnotes in the relevant risk assessments.

After each assessment, the mushroom has been allocated by the risk managers in the project to one of the lists 1–4, see Chapter 2, depending on, whether it is recommended for commercial trade (list 1 and 2) or not (list 3 and 4).

Agaricus abruptibulbus (Peck) Kauffm. s.auct

See *Agaricus* species, other yellowing.

Agaricus arvensis Schaeff

Horse Mushroom (DK: Ager-Champignon, FI: Pelttoherkkusieni, IS: Mókempa, NO: Åkersjampinjong, SE: Snöbollschampinjon).

Background and risk assessment

Horse Mushroom (*Agaricus arvensis*) is generally regarded as edible and good.

It grows saprotrophically in meadows, pastures and garden lawns in summer to autumn and more rarely in coastal, saline meadows. The mushroom sometimes appears in fairy rings. The mushroom is common in Denmark and large parts of Norway, Sweden and Finland, occasional in subarctic regions of Iceland, and is rare in subarctic and alpine areas of Fennoscandia (Knudsen & Vesterholt, 2012).

Occasionally, Horse Mushroom is cultivated and sold as fresh, e.g. in mixtures with other cultivated, fresh mushrooms like Oyster Mushroom (*Pleurotus ostreatus*) and Shiitake (*Lentinula edodes*).

There are no intoxications reported after consumption of Horse Mushroom. However, Horse Mushroom contains several bioactive constituents:

Bioactive constituents

Phenylhydrazine derivatives: Horse Mushroom may contain large amounts of agaritine (β -N-[γ -glutamyl]-4-(hydroxymethyl)phenylhydrazine). Horse Mushroom collected in the Czech Republic was found to contain 475–1,550 (15 samples, mean content 987) mg agaritine per kg fresh weight (Schulzová *et al.*, 2009), whereas Stijve *et al.* (1986) reported approximately 20–1,850 (8 samples, mean content 650) mg agaritine per kg fresh weight in mushrooms purchased or collected in various European countries or the USA. A structurally related phenylhydrazine derivative, the aldehyde agaritinal (β -N-[γ -glutamyl]-4-(formyl)phenylhydrazine) has also been demonstrated in significant amounts in the mushroom. Chulia *et al.* (1988) isolated approximately 40 mg agaritinal per kg fresh weight of Horse Mushroom (Chulia *et al.*, 1988). Using a semi-quantitative thin-layer chromatography method, Stijve and Pittet (2000) found dried Horse Mushroom to contain between 0.5 and 1.5% agaritinal, corresponding to a level between 500–1,500 mg per kg fresh weight.

Horse Mushroom also contains another type of phenylhydrazine derivatives, schaefferals, which are hydrazones chemically formed by a reaction between 4-(formyl)phenylhydrazine and aromatic aldehydes. Three schaefferals were isolated in amounts of about 10 mg per kg fresh mushroom (Kileci-Ksoll *et al.*, 2010).

The structural formulae of agaritine, agaritinal and the three schaefferals are shown below.

Agaritine

Agaritinal

Schaefferals

R=H: Schaefferal A; R=OH: Schaefferal B; R=Unknown: Schaefferal C

Agaritine, or at least its metabolites, are indicated to give rise to tumours in mice by a genotoxic mechanism. Therefore, it cannot be excluded that consumption of Horse Mushroom, which contains large amounts of agaritine and smaller amounts of the structurally related agaritinal, constitutes a cancer risk to humans (for further information see risk assessment of Button Mushroom, *Agaricus bisporus*). Neither agaritinal, nor the schaefferals have been studied for their potential toxicity and carcinogenicity, but at least agaritinal may be anticipated to give rise to similar metabolites as agaritine.

Cadmium: Horse Mushroom belongs to a group of yellowing *Agaricus* species ("Flavescentes"), which have an anise or bitter almond-like smell and become yellowish when rubbed or bruised. In contrast to reddish *Agaricus* species ("Rubescentes"), e.g. Button Mushroom (*A. bisporus*) and Scaly Wood Mushroom (*A. langei*), which may become reddish (or brownish) when cut or rubbed, the yellowing *Agaricus* species bioaccumulate the highly toxic metal cadmium. It has been shown that the cadmium content of *Agaricus* species from the "Rubescentes" group often is at least an order of magnitude lower than the content in the "Flavescentes" group (Laub *et al.*, 1977; Lodenius *et al.*, 1981; Mowitz, 1980; Seeger, 1978; 1982; Woggon & Bickerich, 1978). The bioaccumulation is apparently due to some low molecular weight cadmium-binding proteins, specific for the yellowing *Agaricus* species (Kruse & Lommel, 1979; Meisch *et al.*, 1983; Meisch & Schmitt, 1986). It has been proposed that the bioavailability of cadmium from the yellowing *Agaricus* species is low due to its binding to these specific proteins in the "Flavescentes" group mushrooms (Schellmann *et al.*, 1980; 1984) but feeding studies in rodents have subsequently demonstrated that there is no difference in cadmium bioavailability depending on whether the compound in the diet comes from mushrooms containing the cadmium accumulating

compounds or from cadmium added to the diet in equivalent amounts in the form of cadmium chloride. This has been shown for Wood Mushroom (*A. sylvicola*) fed to mice (Seeger *et al.*, 1986), for Horse Mushroom (*A. arvensis*) fed to rats (Gry *et al.*, 1987; Hansen *et al.*, 1987) and for The Prince (*A. augustus*) fed to mice (Lind *et al.*, 1995).

Cadmium is a highly toxic metal with a very long half-life in humans, ranging from 10 to 30 years. It is primarily toxic to the kidney, where it accumulates and may cause renal dysfunction, which may progress to renal failure. It can also cause bone demineralisation. A health based guidance value for cadmium, a Tolerable Weekly Intake (TWI) of 2.5 microgram cadmium per kg body weight has been established by the European Food Safety Authority (EFSA), based on the renal tubular effects (EFSA, 2009; 2011). EFSA estimates that the mean weekly dietary exposure to cadmium in Europe is 2.3 microgram per kg body weight and that regular consumers of wild mushrooms have a higher dietary exposure 4.3 microgram per kg body weight per week (EFSA, 2009, 2012). Although the current dietary exposure is unlikely to cause adverse renal effects in European consumers, EFSA concludes there is a need to reduce the exposure to cadmium because of the very small safety margin (EFSA, 2012). Furthermore, WHO's International Agency for Research on Cancer has concluded "There is *sufficient evidence* in humans for the carcinogenicity of cadmium and cadmium compounds" and "There is *sufficient evidence* in experimental animals for the carcinogenicity of cadmium compounds" (IARC, 1993; 2012).

Horse Mushroom contains from 0.1 to more than 20 mg cadmium per kg fresh weight, with mean values from 0.4 to 3.5 mg per kg fresh weight (Andersen *et al.*, 1982; Meisch *et al.*, 1977; 1979; Mowitz, 1980; Stijve & Besson, 1976).

In order to protect public health, the European Commission has set limits for cadmium in a series of food items, including a maximum limit of 1.0 mg per kg fresh weight for mushrooms, except for the most frequently traded mushrooms Button Mushroom (*Agaricus bisporus*), Oyster Mushroom (*Pleurotus ostreatus*) and Shiitake (*Lentinula edodes*) for which a limit of 0.20 mg per kg fresh weight has been set (EU Commission, 2006).

Sterner *et al.* (1982) tested an extract of Horse Mushroom in the Ames test using the *Salmonella typhimurium* strains TA98, TA100 and TA2637. The extract was weakly positive in the TA98 and TA100 strains. The activity was not enhanced in the presence of microsomal enzymes. Unfortunately, Sterner *et al.* (1982) give no information, whether they had controlled for the potential presence of histidine in the extract. It is

well known that false positives may be obtained in the presence of histidine. Thus, there is no strong indication that extracts of the Horse Mushroom are mutagenic.

Recommendation

- As Horse Mushroom (*Agaricus arvensis*) efficiently bioaccumulates cadmium and as the amount varies considerably, the content of this toxic and carcinogenic metal should be regularly controlled.
- Due to the potentially high levels of phenylhydrazine derivatives and cadmium, Horse Mushroom should not be eaten in larger amounts (see *A. bisporus* (Button Mushroom) risk assessment).

Listing 1 (as cultivated)

Listing 2 (as wild growing)

Database search information

SciFinder by April 2012. Keywords: *Agaricus arvensis*, agaritine. PubMed by May 2012. Keywords: *Agaricus* and cadmium.

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Agaricus augustus Fr. (*A. perrarus* Schulzer)

The Prince (DK: Prægtig Champignon, FI: Veriherkkusieni, IS: No Icelandic name, NO: Kongesjampinjong; SE: Kungschampinjon).

Background and risk assessment

The Prince (*Agaricus augustus*)⁵ is generally regarded as edible and very good.

It grows saprotrophically in coniferous and deciduous forests, gardens and parks. It is occasional in Denmark, and southern parts of Norway and Sweden, but rare in Finland and in the middle boreal and sub-arctic areas of Norway and Sweden (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of The Prince. However, The Prince contains several bioactive constituents:

⁵ *Agaricus perrarus* is now considered as a synonym for *A. augustus* (Index Fungorum/Species Fungorum (2012) and Knudsen & Vesterholt (2012)).

Bioactive constituents

Phenylhydrazine derivatives: The Prince may contain large amounts of agaritine (β -N-[γ -glutamyl]-4-(hydroxymethyl)phenylhydrazine. The Prince collected in the Czech Republic was found to contain very large amounts of agaritine, 2,260–7,550 mg/kg fresh weight (6 samples, mean content 4,000 mg/kg fresh weight; Schulzová *et al.*, 2009), whereas, *A. perrarus*⁵ contains levels from 841 to 1,170 (2 samples, mean content of 1,000) mg per kg fresh mushroom (Schulzová *et al.*, 2009). Stijve and co-workers have reported lower levels: 0.10–2.20% on dry matter, corresponding to, approximately 100–2,200 (6 samples, mean content 860) mg/kg fresh weight (Stijve *et al.*, 1986) or when analysed by a semi-quantitative thin-layer chromatography (TLC)-method between 1,500–2,500 mg/kg fresh weight (Stijve & Pittet, 2000). A structurally related phenylhydrazine derivative, the aldehyde agaritinal (β -N-[γ -glutamyl]-4-(formyl)phenylhydrazine) has also been demonstrated in The Prince. Using a semi-quantitative TLC-method Stijve & Pittet (2000) reported 0.5–1.5% of the dried mushroom to be agaritinal, which would correspond to a level between 500 and 1,500 mg per kg fresh weight.

The structural formulae of agaritine and agaritinal are shown below.

Agaritine, or at least its metabolites, are indicated to give rise to tumours in mice by a genotoxic mechanism. Therefore, it cannot be excluded that consumption of The Prince, which contain very large amounts of agaritine and the structurally related agaritinal may constitute a risk to humans (for further information see risk assessment of Button Mushroom, *Agaricus bisporus*).

Cadmium: The Prince belongs to the group of yellowing *Agaricus* species ("Flavescentes"), which have an anise or bitter almond-like smell and become yellowish when rubbed or bruised. In contrast to reddish *Agaricus* species ("Rubescentes"), e.g. Button Mushroom (*A. bisporus*)

and Scaly Wood Mushroom (*A. langei*), which may become reddish (or brownish) when cut or rubbed, the yellowing *Agaricus* species bioaccumulates the highly toxic metal cadmium. It has been shown that the cadmium content of *Agaricus* species from the “Rubescentes” group often is at least an order of magnitude lower than the content in the “Flavescentes” group (Laub *et al.*, 1977; Lodenius *et al.*, 1981; Mowitz, 1980; Seeger, 1978; 1982; Woggon & Bickerich, 1978). The bioaccumulation is apparently due to some low molecular weight cadmium-binding proteins, specific for the yellowing *Agaricus* species (Kruse & Lommel, 1979; Meisch *et al.*, 1983; Meisch & Schmitt, 1986). It has been proposed that the bioavailability of cadmium from the yellowing *Agaricus* species is low due to its binding to these specific proteins in the “Flavescentes” group mushrooms (Schellmann *et al.*, 1980; Schellmann *et al.*, 1984) but feeding studies in rodents have subsequently demonstrated that there is no difference in cadmium bioavailability depending on whether the compound in the diet comes from mushrooms containing the cadmium accumulating compounds or from cadmium added to the diet as equivalent amounts of cadmium chloride. This has been shown for Wood Mushroom (*A. sylvicola*) fed to mice (Seeger *et al.*, 1986), for Horse Mushroom (*A. arvensis*) fed to rats (Gry *et al.*, 1987; Hansen *et al.*, 1987) and for The Prince (*A. augustus*) fed to mice (Lind *et al.*, 1995).

Cadmium is a highly toxic metal with a very long half-life in humans, ranging from 10 to 30 years. It is primarily toxic to the kidney where it accumulates and may cause renal dysfunction, which may progress to renal failure. It can also cause bone demineralisation. A health based guidance value for cadmium; a Tolerable Weekly Intake (TWI) of 2.5 microgram cadmium per kg body weight has been established by the European Food Safety Authority (EFSA), based on the renal tubular effects (EFSA, 2009; 2011). EFSA estimates that the mean weekly dietary exposure to cadmium in Europe is 2.3 microgram per kg body weight and that regular consumers of wild mushrooms have a higher dietary exposure, around 4.3 microgram per kg body weight per week (EFSA, 2009; 2012). Although the current dietary exposure is unlikely to cause adverse renal effects in European consumers, EFSA concludes there is a need to reduce the exposure to cadmium because of the very small safety margin (EFSA, 2012). Furthermore, WHO’s International Agency for Research on Cancer (IARC) has concluded that “There is *sufficient evidence* in humans for the carcinogenicity of cadmium and cadmium compounds” and “There is *sufficient evidence* in experimental animals for the carcinogenicity of cadmium compounds” (IARC, 1993; 2012).

The Prince contains 0.5–11 mg cadmium per kg fresh weight (18 samples), with a mean value around 4 mg per kg mushroom (Andersen *et al.*, 1982; Meisch *et al.*, 1977, 1979; Mowitz, 1980; Seeger, 1978; Stijve & Besson, 1976). Lind *et al.* (1995) reported a level of more than 80 mg cadmium per kg dried mushroom corresponding to more than 8 mg cadmium per kg fresh weight.

In order to protect public health, the European Commission has set limits for cadmium in a series of food items, including a maximum limit of 1.0 mg per kg fresh weight for mushrooms, except for the most frequently traded mushrooms Button Mushroom (*Agaricus bisporus*), Oyster Mushroom (*Pleurotus ostreatus*) and Shiitake (*Lentinula edodes*) for which a limit of 0.20 mg per kg fresh weight has been set (EU Commission, 2006).

Recommendation

- As The Prince (*Agaricus augustus*) efficiently bioaccumulates cadmium, and the amounts varies considerably, the content of this toxic and carcinogenic metal should be regularly controlled.
- Due to the potentially high levels of phenylhydrazine derivatives and cadmium, The Prince should not be eaten in larger amounts (see *A. bisporus* (Button Mushroom) risk assessment).

Listing 2

Database search information

SciFinder by February 2012. Keywords: *Agaricus augustus* or *Agaricus per-rarus* or agaritine PubMed by May 2012. Keywords: *Agaricus* and cadmium.

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Agaricus bisporus (J.E. Lange) Imbach (*A. hortensis* (Cooke) S. Imai, *A. brunnescens* Peck)

Cultivated Mushroom (Button Mushroom) (DK: Have-Champignon, (Hvid Have-Champignon, Brun Have-Champignon, "Champignon", "Portobello"), FI: Viljelyherkkusieni, IS: Matkempa (matkempingur), NO: Dyrket sjampinjong ("Aromasopp", "Portobello"), SE: Trädgårdschampinjon (Odlade former av Trädgårdchampinjon, Vit Trädgårdschampinjon, Brun Trädgårdschampinjon, "Champinjon", "Portobello").

Background and hazard identification

Cultivated Mushroom (Button Mushroom) (*Agaricus bisporus*) is the most commonly cultivated and consumed mushroom. In 1997, the year from which the latest world production figures were found, it was cultivated in 2 million tons (Chang & Miles, 2004). Several different varieties/forms can be found on the market, for example varieties with smaller white or brown caps (buttons) and those with larger brown caps ("Portobello"). Button Mushroom is commercially available as fresh, canned, dried, or otherwise processed.

In the wild, Button Mushroom is native to grasslands in Europe and North America. It is occasionally found from summer to autumn in Denmark, and in hemiboreal southern parts of the Nordic countries. It is likely that Button Mushroom found in the nature often originates from

mycelia established from waste from cultivated specimens (Knudsen & Vesterholt, 2012).

No acute intoxications are known after consumption of Button Mushroom, except for very rare cases of allergic reactions (with potential cross-reactivity to moulds and spinach) (Pelzer, 2000; Dauby *et al.*, 2002; Hegde *et al.*, 2002; Herrera *et al.*, 2002; Venkatesh & Hegde, 2003; Hegde & Venkatesh, 2004; Herrera-Mozo *et al.*, 2006; Ho & Hill, 2006).

As allergy was not dealt with in the Nordic report (Andersson & Gry, 2004) being the background for the present risk assessment, the few cases of food allergy known are described in the hazard characterization. Mushroom growers disease, related to the cultivation of Button Mushroom, is described in Volume II, section 1, Annex V.

Between the early 1960s and the middle of the 1980s phenylhydrazine derivatives were identified in the Button Mushroom. These findings raised concern as the majority of all known hydrazines are genotoxic and carcinogenic agents. The concern was confirmed in subsequent carcinogenicity studies in mice. The potential human cancer risk was addressed in three Nordic reports on phenylhydrazines in the Button Mushroom (Gry & Pilegaard, 1990/1991; Gry & Andersson, 1998 and Andersson & Gry, 2004). The latest mentioned report stated, that it cannot be excluded that consumption of Button Mushroom constitutes a cancer risk for the consumer.

The most abundant phenylhydrazine derivative in Button Mushroom is agaritine (β -N-[γ -L(+)-glutamyl]-4-(hydroxymethyl)phenylhydrazine), which usually occurs in quantities between 200 and 400 mg per kg in the fresh mushroom; sometimes levels as low as 80 mg per kg or as high as 1,700 mg per kg have been found. One of the anticipated metabolites of agaritine, the 4-(hydroxymethyl)phenyldiazonium ion (HPD), is believed to be the most potent phenylhydrazine derivative in Button Mushroom but it occurs at low levels, between 0.6 and 4 mg per kg fresh weight. The other phenylhydrazine derivatives in the mushroom are 4-(carboxy)phenylhydrazine (CPH; 10–11 mg per kg fresh weight) and β -N-[γ -L(+)-glutamyl]-4-(carboxy)phenylhydrazine (GCPH; 16–42 mg per kg fresh weight) (Andersson & Gry, 2004).

More recent studies have confirmed these observations, reporting levels between 200 and 1,800 mg agaritine per kg fresh weight of the mushroom (Kondo *et al.*, 2006a, 2006b, Nagaoka *et al.*, 2006; Koge *et al.*, 2011). However, Nagaoka *et al.* (2006) did not find CPH in the Button Mushroom. Sommer *et al.* (2009) reported around 1,500 mg agaritine per kg fresh *Agaricus bisporus*. The spores from Button Mushroom have been shown to contain in average 3,040 mg agaritine per kg of spores (Janák *et al.*, 2005).

The structural formulae of the phenylhydrazine derivatives in the Button Mushroom and one of the potential metabolites, 4-(hydroxymethyl)phenylhydrazine (HPH) are shown below.

Agaritine

4-(Hydroxymethyl)phenyldiazonium (HPD)

4-(Carboxy)phenylhydrazine (CPH)

β-N-(γ-L(+)-Glutamyl)-4-(carboxy)phenylhydrazine (GCPH)

4-(Hydroxymethyl)phenylhydrazine (HPH)

The body of the text that follows is based on data and conclusions from the Nordic report: “Phenylhydrazines in the Cultivated Mushrooms (*Agaricus bisporus*)” by Andersson & Gry (2004). Newer data which have been published after this report are highlighted and referenced, and contribute to this updated risk assessment.

Hazard characterisation

The Nordic report presented the information available at the time regarding absorption and metabolism of agaritine, the acute, sub-chronic and developmental toxicity as well as DNA-binding, genotoxicity, and carcinogenicity of agaritine and other phenylhydrazines occurring in the Button Mushroom. In addition carcinogenicity data on mice fed raw, baked or cooked Button Mushrooms were reviewed. This information is briefly summarized below together with more recent data; more detailed information can be found in the Nordic report (Andersson & Gry, 2004). In addition, available information on the allergenicity of the Button Mushroom is presented.

Allergenicity

A few cases of allergenicity due to the Button Mushroom have been described during the last decade. Hegde *et al.* (2002) described a severe case of anaphylaxis in an Indian woman who experienced facial oedema and generalized urticaria minutes after eating Button Mushroom curry. Skin prick tests with mushroom extracts prepared from raw, cooked, steamed or dried mushrooms were strongly positive, but surprisingly no mushroom specific IgE could be detected by enzyme-linked immunosorbent assay. Separation of mushroom constituents and testing each fraction in skin prick tests revealed that the allergen was mannitol, a sugar alcohol with very low molecular weight (<1 kDa). Subsequently,

Hegde and Venkatesh (2004) identified mannitol-specific human IgE and showed that they were specific to the D-isomer of mannitol. As mannitol is inert and cannot react with proteins to form hapten-carrier conjugates and elicit immune response, Venkatesh and Hegde (2003) hypothesised an alternative mechanism for the allergenicity. It was suggested that D-mannose reacts *in vivo* with exposed amino groups of proteins, forming Schiff base intermediates bearing a D-mannitoyl moiety, which closely resembles D-mannitol. The intermediate elicits the formation of mannitol-specific IgE in susceptible individuals. Once an individual is sensitized with the formation of mannitol-specific IgE, mannitol can cause anaphylactic reactions by acting either as a univalent anaphylactogen or a bivalent hapten.

Ho and Hill (2006) reported a person being allergic to the Button Mushroom (and the Field Mushroom, *Agaricus campestris*). Dishes containing these mushrooms as well as cooking vapours from processing resulted in discomfort in the mouth, itchy ears, nausea, diaphoresis and discomfort in the throat. Skin prick tests with *Agaricus* extracts gave positive reaction. Two other cases of allergy to Button Mushroom were described by Pelzer (2000). In both these cases symptoms including abdominal pain developed after intake of cooked mushrooms. Dauby *et al.* (2002) studied the antigenic cross-reactivity between Button Mushroom and mould spores (positive skin testing to moulds) in a patient with oral allergy symptoms to raw but not to cooked Button Mushroom. Skin prick tests with extracts of the raw Button Mushroom and four different moulds, and immune-blot assays revealed IgE antibodies directed against similar weight proteins (43 and 67 kDa) in the raw mushroom and three of the four moulds. These antigens were absent in the cooked mushroom.

A case of cross-reactivity resulting in food allergy to spinach and mushrooms has been described in a woman with persistent allergic asthma to airborne moulds (Herrera *et al.*, 2002). Antibodies in the serum recognized a 22 kDa large protein in extracts of the Button mushroom. Co-administration of a spinach extract or a mould extract inhibited this reaction. Subsequently the same investigators screened a group of atopic patients allergic either to moulds (n=33) or to mites and/or pollen (n=13) by immuno-blotting extracts from three moulds and the Button Mushroom. The analysis of the data revealed a link between allergenicity to moulds and positive skin prick tests with extracts from the Button Mushroom and/or spinach. Seven patients allergic to moulds had a strong recognition of a protein with a molecular weight of about 30 kDa present both in spinach and mushroom extracts. The investigators concluded that a novel

cross-reactive allergen between aeroallergens and foods had been identified (Herrera-Mozo *et al.*, 2006).

It is concluded that only a few cases of allergy due to Button Mushroom consumption have been described. It has also been established that cross reactivity between Button Mushroom and spinach or moulds is possible.

Absorption and metabolism

Studies in experimental animals (rodents) using radio-labelled agaritine have shown that the compound and/or its degradation products are rapidly absorbed from the gastrointestinal tract. Blood levels of radioactivity were shown to peak within a few hours. No major qualitative differences in the pharmacokinetic behaviour of agaritine in rat and mouse have been observed, and, furthermore, the metabolic profile of agaritine seems to be the same in both species.

Recently Kondo and co-workers (2006a; 2008) noted that plasma levels of agaritine as determined by a sensitive LC/MS/MS method peaked at around 20 minutes after oral dosing of mice and then returned to basal levels at around 100 minutes. At the highest dose tested (40 mg per kg body weight), the maximum concentration was 0.37 mg/L, the time to the maximum concentration 0.33 h, and the plasma half-life of agaritine 0.71 h. These investigators were also able to detect *in vitro* the metabolite HPH after incubation of agaritine with γ -glutamyltransferase. This enzyme is present in various tissues of the body and is believed to cleave agaritine into L-glutamic acid and HPH. The latter compound is subsequently transformed to HPD that is believed to produce reactive radical species able to damage macromolecules.

Acute and sub-chronic toxicity

Very little is known about the potential acute and sub-chronic toxicity of the phenylhydrazines occurring in the Button Mushroom. According to the few and poor studies available, CPH and its γ -glutamyl derivative GCPH are significantly less toxic than other phenylhydrazines occurring in the mushroom.

Nieminen and co-workers (2009) have published a study on the potential myo- and hepatotoxic effects of different cultivated mushrooms in mice. The animals (six females per group) were for 5 days supplied large quantities of dried and powdered cultivated mushrooms in the feed. One of the cultivated mushrooms, Button Mushroom, was given as 1.8%, 3.6% or 5.4% of the feed. The feeding resulted in exposures corresponding to 24, 46 and 69 g, respectively, which is equivalent to 1.7, 3.2 or 4.9 kg per person (70 kg) per day, or when corrected for body surface constant 141

g, 270 g or 404 g per person (70 kg) per day, respectively, of fresh Button Mushroom. All mice were healthy at the end of the study. Of the many clinical parameters studied, plasma bilirubin (indicator of liver damage) was significantly increased in a dose dependent manner. As creatine kinase (indicator of myopathy) was significantly increased only at the lowest dose level, this observation is likely to have been incidental. No histological pathological changes in studied organs were found.

Developmental toxicity

There are no indications that Button Mushroom or the phenylhydrazines occurring in the mushroom are embryotoxic or teratogenic in mammals at biologically relevant doses. However it should be stressed, that the design of the few studies available did not follow modern guidelines for these types of studies.

DNA-binding and genotoxicity

A low frequency of DNA damage has been observed in various tissues of experimental animals exposed orally to agaritine or HPD, observations indicating that these compounds may be genotoxic and possibly carcinogenic. This interpretation is supported by the observation that Button Mushroom extracts, as well as agaritine and the other phenylhydrazines occurring in this mushroom slightly increase the mutation frequency in bacterial test systems *in vitro*.

Recent *in vivo* studies in mice have revealed that the oxidative stress marker 8-OHdG increased significantly in the urine of agaritine treated mice (Kondo *et al.*, 2008). Furthermore, in *in vitro* studies on the mechanism of action of the potential carcinogen CPH, Oikawa *et al.* (2006) showed that the compound induced DNA damage via hydrogen peroxide formation (oxidative damage) and DNA adducts via a Cu(II)-dependent reaction, the latter possibly via phenyl-radical forming adducts with adenine and guanine. These new studies, as well as the observation that agaritine induces gene mutations in transgenic mice, and that HPD induces micronuclei in peripheral lymphocytes (Andersson & Gry, 2004) indicate that DNA lesions of potential importance for tumour induction may be formed by the phenylhydrazines in the Button Mushroom.

Carcinogenicity

Nine out of twelve carcinogenicity studies with the Button Mushroom or its phenylhydrazine constituents have shown carcinogenic effect in Swiss albino mice, whereas two studies in rats did not increase the tumour level compared to the controls.

Studies in mice: In 3 out of 4 *peroral* long-term studies tumours were induced in various tissues of mice fed raw, baked, or freeze-dried Button Mushroom. In the study with raw mushrooms an estimated average daily exposure of 4.7 g mushroom per mouse gave rise to a 38% increase in forestomach tumours (Toth & Erickson, 1986). With baked mushrooms an exposure of 5.6 g mushrooms per mouse gave rise to a 20% increase in glandular stomach tumours (Toth *et al.*, 1997a). With the freeze-dried mushrooms an exposure of 12 g freeze-dried mushrooms per kg body weight per day in mouse gave rise to a 22% increase in lung tumours (Toth *et al.*, 1998). In a fourth study with baked mushrooms which had another (more balanced) feeding schedule, the increase in tumour incidence was not significant (Toth *et al.*, 1997b).

Three of the four phenylhydrazines known to occur in Button Mushroom, CPH, GCPH, and HPD, were carcinogenic at high doses in Swiss albino mice when administered orally by gavage or in drinking water (and also when injected subcutaneously). In the study with CPH an estimated average daily exposure of 204 mg CPH per kg body weight gave rise to a 38% increase in tumours of the aorta and large arteries (McManus *et al.* 1987). With GCPH an exposure of 149 mg GCPH per kg body weight gave rise to a 26% increase in tumours in the subcutaneous tissue (Toth, 1986). With HPD an exposure of 0.50 mg per kg body weight per day gave rise to a 32% increase in glandular stomach tumours (Toth *et al.*, 1982).

The fourth phenylhydrazine, agaritine, which is the most abundant one in Button Mushroom, was not found to be carcinogenic when given in drinking water or by subcutaneous injection (Toth *et al.*, 1981; Toth & Sornson, 1984).

However, no precautions were taken in these studies with agaritine to protect against oxidative degradation of agaritine in water. Agaritine has been shown to be very vulnerable to such degradation in aqueous solution (Hajslova *et al.* 2002). The low stability of agaritine in water has also recently been shown by Koge and colleagues (2011). They showed that pure agaritine in aqueous solution is very unstable at 120 °C and decompose exponentially. Around 73% of the agaritine in an extract from the Button Mushroom was destructed after 30 min of heating at 120 °C.

It should be stressed that none of the long term carcinogenicity studies in mice with Button Mushroom, or the phenylhydrazines occurring in the mushroom, have been performed according to approved standard protocols for carcinogenicity studies. Thus, all cancer studies in mice have been performed only with out-bred Swiss albino mice in a single laboratory. Furthermore, the feeding regimen of the mice with mushrooms was not considered balanced and animals were exposed to the compounds/mushrooms until they died or were found in poor condition instead of for a predetermined length of time. In addition, the control groups were usually not started at the same time as the treated groups. Although at least three dose levels are recommended in carcinogenicity studies in order to identify potential dose-response effects, this requirement was only fulfilled in one study.

Studies in rats: There was no significant increase in tumour frequencies in two long-term studies in rats, but in these rather inadequate studies the number of animals was too low to detect a small increase in tumour incidence. Furthermore, the processing (pre-treatment) of the mushrooms used in the rat studies (ordinary drying and milling, or washing, combined with pressure cooking, homogenising, and mixing with tap water) has later been shown to result in a significant degradation of the potentially active phenylhydrazines in Button Mushroom (Hajslova *et al.*, 2002; Andersson & Gry, 2004; Koge *et al.* 2011).

In a more recent rat study in F344 rats Lee and colleagues (2008) investigated the carcinogenicity of another *Agaricus* species (*A. subrufescens*; syn. *A. blazei*) also known to contain agaritine. A diet containing powder of lyophilized aqueous extract of the mushroom was fed for 2 years to the rats. There was no increase in tumours compared to controls. This rat study is of low relevance for the evaluation of potential carcinogenicity of the Button Mushroom, as the agaritine content in the rat feed was not analysed, and, most importantly, *A. subrufescens* is another species than *A. bisporus* and is claimed to contain anti-cancer constituents.

IARC, status: In 1983 WHO's International Agency for Research on Cancer concluded that "There is limited evidence of the carcinogenicity of the derivatives of two fungal metabolites of agaritine in experimental animals. In the absence of epidemiological data, no evaluation of the carcinogenicity of agaritine to humans could be made" (IARC, 1983) and in 1987 Group 3 (IARC, 1987) "not classifiable as to its carcinogenicity to humans."

Roupas et al. statement: Recently, Roupas *et al.* (2010) concluded in "Mushrooms and agaritine" that "critical analysis of the studies to date leads to the conclusion that consumption of *A. bisporus* and other edible mushrooms does not result in any known toxicological or carcinogenic

effects in healthy humans.” Although these investigators have considered some of the studies referred to in the Nordic report “Phenylhydrazines in the Cultivated Mushroom (*Agaricus bisporus*)” and in the present risk assessment, they mainly base their conclusion on clinical trials lasting up to one year on patient groups with different diseases using other mushroom species than Button Mushroom. Such studies are not very relevant for the estimation of potential human cancer risk due to consumption of Button Mushroom.

Although it is difficult to conclude on the carcinogenic potential of Button Mushroom due to the shortcomings in the way the carcinogenicity studies in rodents have been performed, the comprehensive set of data on raw and on processed Button Mushroom and on the phenylhydrazine derivatives occurring in the mushroom indicates that this mushroom is carcinogenic in mice.

Exposure assessment

The estimated annual *per capita* intake of Button Mushroom (*Agaricus bisporus*) varies between the Nordic countries. Based on data available in 2004 it was 0.6 kg in Finland, 1.7 kg in Norway, 2.1 kg in Iceland, 2.2 kg in Denmark and 2.4 kg in Sweden. The proportion of the purchased Button Mushroom being the fresh mushroom varied between 33 and 70% among the countries, the rest being preserved, mainly canned, mushrooms (Andersson & Gry, 2004).

Since the *per capita* intake is an estimation of average intake and does not take into consideration that mushrooms usually are consumed only by a part of the population, the *per capita* consumption is too low to express the consumption among mushroom eating consumers. For instance Danish data, based on a limited consumer study, indicate that only around 50% of the population consumes Button Mushroom, and that 5% of the population consumes five times the median intake and 0.1% thirty times the median intake (Andersson & Gry, 2004).

As mentioned in the hazard identification, the potentially toxic compounds in Button Mushroom are the phenylhydrazine derivatives. Agaritine occurs in quantities between 80 and 1,800 mg per kg fresh weight (usually between 200 and 400 mg/kg fresh mushroom). The anticipated metabolite of agaritine, HPD, occurs at low levels (0.6–4 mg per kg fresh weight). The other phenylhydrazine derivatives in the mushroom are CPH (10–11 mg per kg fresh weight) and GCPH (16–42 mg/kg fresh weight) (Andersson & Gry, 2004; Koge *et al.*, 2011; Kondo *et al.*, 2006a; b; Nagaoka *et al.*, 2006).

Data on the influence of food processing on the amounts of phenylhydrazines in the consumed mushroom are scanty. Properly freeze-dried mushrooms seem to contain similar agaritine levels as fresh mushrooms, whereas ordinary frozen and then thawed, boiled, microwave heated, and fried mushrooms contain reduced levels (Schulzova *et al.*, 2002; Andersson & Gry, 2004). Canned mushrooms only contain up to 10% of the agaritine level in fresh mushrooms (Andersson & Gry, 2004; Kondo *et al.*, 2006b).

Taken together these data allow a rough estimation of the daily *per capita* exposure to these phenylhydrazines in the Nordic countries. Agaritine is used as a representative of these phenylhydrazines. With a content of 200–400 mg agaritine per kg fresh non-processed Button Mushroom the daily *per capita* intake of agaritine in Finland, having the lowest Button Mushroom intake in the Nordic countries, would be 0.33–0.66 mg, whereas the daily *per capita* intake by a consumer in Sweden, having the highest intake of Nordic countries, would be 1.3–2.6 mg agaritine. Consumers having preference for Button Mushroom would be exposed to considerably higher amounts of agaritine. However, as processing reduces the level of agaritine in the fresh Button Mushroom, average intake estimates based on the raw mushroom would be a worst case scenario.

Risk characterisation

Carcinogenicity. As described in the hazard characterization it has been experimentally shown that Button Mushroom and some of its phenylhydrazine constituents may give rise to DNA damage of concern, e.g. mutations of relevance for the development of tumours. This indicates that the carcinogenic effects observed in mice fed raw mushrooms, processed mushrooms or the various phenylhydrazines found in the mushroom have been induced by a genotoxic mechanism. For genotoxic and carcinogenic compounds it is generally believed that even low exposures may constitute a cancer risk. The results of the carcinogenicity studies in mice and the estimated information on the exposures were used for the following quantitative risk assessment of cancer induction from consumption of Button Mushroom in the Nordic countries.

Human exposure. The estimation of the human exposure of Button Mushroom (*per capita* intake) is based on information on production data. The lowest intake was registered for Finland (0.6 kg/person/year), whereas the other Nordic countries on average consumed around 2.1 kg/person/year.

However, these consumption estimates suffer from a number of shortcomings. It is for instance indicated, as mentioned above, that, around half of the Danish adult population claims not to consume Button Mushroom, 5% eat more than five times the median intake and 0.1% thirty times more than the median intake. This means that sub-populations of “high” consumers in the Danish example would have maybe 5 or 30 times higher cancer risks than the average consumer. As described in the hazard characterization, Button Mushroom may contain levels of agaritine between 80 and 1,800 mg per kg fresh mushroom (usually 200–400 mg per kg), whereas the levels of the other phenylhydrazine derivatives are lower: HPD between 0.6 and 4 mg per kg fresh weight, CPH 10–11 mg per kg fresh weight and GCPH 16–42 mg per kg fresh weight. These data only allow a rough estimation of the average daily intake of the various mushroom phenylhydrazines by the consumers.

Exposure levels in carcinogenicity studies. As given by the hazard characterization, the carcinogenicity study with the raw Button Mushroom in mice resulted in an estimated average daily exposure of 4.7 g mushrooms per mouse and gave rise to a 38% increase in forestomach tumours. The carcinogenicity study with baked Button Mushroom resulted in an exposure of 5.6 g mushrooms per mouse per day and gave rise to a 20% increase in glandular stomach tumours. The study with freeze-dried mushrooms led to an average exposure of 12 g freeze-dried mushrooms per kg body weight per day and resulted in a 22% increase in lung tumours. Three of the four phenylhydrazines known to occur in Button Mushroom – CPH, GCPH, and HPD – were carcinogenic in mice at high doses when administered orally by gavage or in drinking water. In the study with CPH an estimated average daily exposure of 204 mg CPH per kg body weight gave rise to a 38% increase in tumours of the aorta and large arteries. With GCPH an exposure of 149 mg GCPH per kg body weight gave rise to a 26% increase in tumours in the subcutaneous tissue, and with HPD an exposure of 0.50 mg per kg body weight per day gave rise to a 32% increase in glandular stomach tumours. The fourth phenylhydrazine, agaritine, which occurs in largest amount, was not found to be carcinogenic when given in drinking water (or by subcutaneous injection). However no precautions were taken in these studies to protect against oxidative degradation of agaritine in water. Agaritine has more recently been shown to be very vulnerable to such degradation in aqueous solution.

Quantitative risk assessment. Based on the experimental data available from the cancer studies in mice and from human exposure data a quantitative risk assessment was performed. Several assumptions were

made. One was that it is possible to perform a linear extrapolation from the high exposure levels in mice to the lower human exposure levels. Other assumptions included that man and mouse have the same metabolism and sensitivity, that the weight of a mouse is on average 25 g and that of a human 70 kg, and that the survival of animals exposed to the tested compound/food is comparable to the survival of the control animals. Furthermore, the influence of processing on phenylhydrazine content of mushrooms was not taken into consideration.

Based on the studies with raw, baked and lyophilized Button mushroom fed to mice and the available information and assumptions made, an estimated life-time cancer risk of approximately 110 to 250 extra cancer cases per one million average consumers in Denmark, Iceland, Norway and Sweden can be calculated. The risk for Finnish average consumers will be lower. A linear extrapolation of the cancer data in mice for the three phenylhydrazines occurring in the Button Mushroom (CPH, GCPH and HPD) would result in risks from 2 to 200 extra cancer cases per million average consumers during lifetime. Overall, a risk of 2–250 extra cancer cases during lifetime per million Nordic average consumers can be estimated.

Uncertainties: There are uncertainties related to the design of the carcinogenicity studies, to the exposure assessments, to the use of linear extrapolation and to the assumptions required to perform the quantitative risk assessment. This results in a very conservative assessment (worst case).

Conclusion: It is indicated that Button Mushroom and some of its phenylhydrazine constituents may give rise to cancer in mice, presumably via a genotoxic mechanism. Therefore it cannot be excluded, that consumption of Button Mushroom may constitute a risk for humans.

Recommendation

As it is concluded from animal and *in vitro* studies that the phenylhydrazine derivatives occurring in Button Mushroom (*A. bisporus*) as well as the mushroom itself may be genotoxic and carcinogenic, a carcinogenic risk for humans cannot be excluded.

It is therefore recommended not to eat Button Mushroom in larger amounts. The average consumption has been estimated to be 1–2 kg per person per year in the Nordic countries (Andersson & Gry, 2004).

Proper processing of the fresh mushroom reduces the amounts of potentially carcinogenic constituents. The fried, microwave-heated, boiled (especially if boiling water is discarded), and canned mushrooms contain significantly less of the potentially carcinogenic phenylhydrazines. Also

ordinary freezing and subsequent thawing (but not freeze-drying) will reduce the content of phenylhydrazines in the mushroom.

It is therefore recommended to process/cook Button Mushroom before consumption.

Listing 1

Database search information

SciFinder by January 2012. Keywords: Agaritine. *Agaricus bisporus*.

PubMed by January 2012: Keywords: Agaritine.

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Agaricus bitorquis (Quél.) Sacc.

Pavement Mushroom (DK: Vej-Champignon, FI: Agaricus lajin herkkusienet, IS: No Icelandic name, NO: Bysjampinjong, SE: Vågchampinjon).

Background and risk assessment

Pavement Mushroom (*Agaricus bitorquis*) is generally regarded as edible and good.

It is a saprotrophic mushroom growing in dry places in cities, along roads, in gardens and parks, typically breaking up through the substrate in late spring to autumn. It is common in Denmark, occasional in southern Finland, Norway and Sweden and rare in middle boreal areas (Knudsen & Vesterholt, 2012).

In some countries outside the Nordic countries Pavement Mushroom (*A. bitorquis*) is preferred for cultivation instead of the Button Mushroom (*A. bisporus*) (Schulzová *et al.*, 2002; 2009).

There are no intoxications reported after consumption of Pavement Mushroom. However, Pavement Mushroom may contain very high levels of agaritine (β -N-[γ -glutamyl]-4-(hydroxymethyl)phenylhydrazine). Pavement Mushroom collected in the Czech Republic was found to contain 216–3,001 mg agaritine per kg fresh weight (11 samples, mean content 1,470 mg/kg fresh weight) (Schulzová *et al.*, 2009), whereas Stijve and co-workers reported approximately 50–2,000 mg agaritine per kg

fresh weight in mushrooms collected in different European countries (5 samples, mean content 716 mg/kg fresh weight; Stijve *et al.*, 1986), or 1.5–2.5% of the dried mushroom being agaritine, corresponding to 1,500–2,500 mg per kg fresh weight (Stijve & Pittet, 2000). It has been noted that the content of agaritine in cultivated strains of Pavement Mushroom is lower than in specimens collected from nature. Thus in a study on cultivated *Agaricus* species Schulzová *et al.* (2002) reported cultivated strains of Pavement Mushroom to contain 249–360 mg agaritine per kg fresh weight.

The structural formula of agaritine is shown below:

Agaritine

Agaritine, or at least its metabolites, are indicated to give rise to tumours in mice by a genotoxic mechanism. Therefore, it cannot be excluded that consumption of Pavement Mushroom, which contains considerable amounts of agaritine, may constitute a cancer risk to humans (for further information, see risk assessment of Button Mushroom, *Agaricus bisporus*).

Pavement Mushroom, like the Button Mushroom (*A. bisporus*) and Scaly Wood Mushroom (*A. langei*), belongs to the group of reddening *Agaricus* species ("Rubescentes") which may become reddish (or brownish), when cut or rubbed. Contrary to the group of yellowing *Agaricus* species ("Flavescentes") Pavement Mushroom and other *Agaricus* species from the "Rubescentes" group, the reddening *Agaricus* species, do not bioaccumulate the toxic metal cadmium (Laub *et al.*, 1977; Lodenius *et al.*, 1981; Mowitz, 1980; Seeger, 1978; 1982).

Recommendation

Pavement Mushroom (*Agaricus bitorquis*):

- Due to the potentially high levels of phenylhydrazine derivatives in Pavement Mushroom, the species should not be eaten in larger amounts (see the risk assessment of Button Mushroom, *Agaricus bisporus*).

Listing 2

Database search information

SciFinder by April 2012. Keywords: *Agaricus bitorquis*.

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Agaricus brunnescens Peck

See *Agaricus bisporus*.

Agaricus campestris L.

Field Mushroom (DK: Mark-Champignon, FI: Nurmiherkkusieni, IS: Túnkempa (Túnkempingur, Túnætisveppur), NO: Beitesjampinjong, SE: Ängschampinjon).

Background and risk assessment

Field Mushroom (*Agaricus campestris*) is generally regarded as edible and good.

It is a saprotrophic mushroom growing in open meadows, grassland, alvar areas and lawns. It is very common in most parts of the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Field Mushroom except for a case reported by Ho and Hill (2006) that showed allergic reactions to Button Mushroom (*A. bisporus*) and Field Mushroom. Dishes containing these mushrooms, as well as cooking vapours during processing, resulted in discomfort in the mouth, itchy ears, nausea, diaphoresis and discomfort in the throat. Skin prick tests with *Agaricus* extracts gave positive reaction.

However, Field Mushroom may contain large amounts of agaritine (β -N-[γ -glutamyl]-4-(hydroxymethyl)phenylhydrazine). Field Mushroom collected in the Czech Republic was found to contain 0–2,230 mg agaritine per kg fresh weight (22 samples, mean content 487 mg/kg

fresh weight) (Schulzová *et al.*, 2009), whereas Stijve and co-workers reported approximately 20–1,000 mg agaritine per kg fresh weight in mushrooms collected in different European countries (11 samples, mean content 260 mg/kg fresh weight; Stijve *et al.*, 1986) or 0.5–1.5% of the dried mushroom to be agaritine, corresponding to between a level of 500 and 1,500 mg/kg fresh weight (Stijve & Pittet, 2000). A structurally related phenylhydrazine derivative, the aldehyde agaritinal (β -N-[γ -glutamyl]-4-(formyl)phenylhydrazine) has also been demonstrated in Field Mushroom in significant amounts. Chuilia *et al.* (1988) reported approximately 40 mg per kg fresh weight and Stijve and Pittet (2000) a level between 50–500 mg per kg fresh weight.

The structural formulae of agaritine and agaritinal are shown below.

Agaritine, or at least its metabolites, are indicated to give rise to tumours in mice by a genotoxic mechanism. Therefore, it cannot be excluded that consumption of Field Mushroom which contains considerable amounts of agaritine and the structurally related agaritinal constitutes a cancer risk to humans (for further information see risk assessment of Button Mushroom, *A. bisporus*). Agaritinal has not been studied for its potential toxic effects, but agaritinal may be anticipated to give rise to similar metabolites as agaritine.

Field Mushroom, like Button Mushroom (*A. bisporus*) and Scaly Wood Mushroom (*A. langei*), belongs to the group of reddening *Agaricus* species (“Rubescentes”) which may become reddish (or brownish), when cut or rubbed. Contrary to the group of yellowing *Agaricus* species (“Flavescentes”), Field Mushroom and other *Agaricus* species from the “Rubescentes” group, the reddening *Agaricus* species, do not bioaccumulate the toxic metal cadmium (Laub *et al.*, 1977; Lodenius *et al.*, 1981; Mowitz, 1980; Seeger, 1978; 1982).

Field Mushroom contains a remarkably thermostable lectin, which is a dimer with a molecular weight of 30.4 kDa. This lectin keeps its hemagglutinating activity during heating up to 90 °C, but the activity is lost at 100 °C. The lectin has shown anti-proliferative effects on hepatoma and breast cancer cells cultured *in vitro* (Zhao *et al.*, 2011). Many lectins influence the digestibility of certain foods especially if the foods are insufficiently pre-treated/cooked. If for instance certain dried kidney beans are not thoroughly pre-treated and cooked, they may give rise to gastrointestinal adverse reactions due to remaining thermostable lectins (Liener, 1983). Also in mushrooms certain lectins are suspected to give rise to adverse reactions (Horibe *et al.*, 2010; Gachet *et al.*, 1996; Wang *et al.*, 1998). However, there is no information available whether the thermostable lectin in Field Mushroom has given rise to intoxications.

Recommendation

Field Mushroom (*Agaricus campestris*):

- Due to the potentially high levels of phenylhydrazine derivatives in Field Mushroom, the species should not be eaten in larger amounts (see the risk assessment of Button Mushroom (*Agaricus bisporus*)).

Listing 2

Database search information

SciFinder by 1 April 2012. Keywords: *Agaricus campestris* or *Agaricus campester*.

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Agaricus essettei Bon

See *Agaricus* species, other yellowing.

Agaricus excellens (F. H. Møller) F. H. Møller

See *Agaricus* species, other yellowing.

Agaricus haemorrhoidarius Schulzer s. J. E. Lange

See *Agaricus* species, other not yellowing.

Agaricus hortensis (Cooke) S. Imai.

See *Agaricus bisporus*.

Agaricus langei (F. H. Møller) F. H. Møller

See *Agaricus* species, other not yellowing.

Agaricus macrosporus (F. H. Møller & Jul. Schäff.)
Pilát non Montagne

See *Agaricus* species, other yellowing.

Agaricus perrarus Schulzer

See *Agaricus augustus*.

Agaricus species, other not yellowing, e.g. *A. langei* (F. H. Møller) F. H. Møller (*A. haemorrhoidarius* Schulzer s. J. E. Lange) and *A. sylvaticus* Schaeff

Agaricus species, other not yellowing (DK: Andre ikke-gulnende champignonner, FI: Muut ei kellertävät *Agaricus* lajin herkkusienet, IS: Aðrar kempur sem ekki gulna við hnjask, NO: Andre ikke gulnende sjampinjonger, SE: Andra icke gulnande *Agaricus* arter).

A. langei

A. sylvaticus

Background and risk assessment

Besides Button Mushroom (*Agaricus bisporus*), Pavement Mushroom (*A. bitorquis*) and Field Mushroom (*A. campestris*) there are other *Agaricus* species that do not yellow when handled. Many of these other species belong to the group of reddishing (“*Rubescentes*”) *Agaricus* species, like the Scaly Wood Mushroom (*A. langei*) and Blushing Wood Mushroom (*A. sylvaticus*), which both are also regarded as edible and collected for consumption.

They grow saprotrophically in coniferous and deciduous forests and parks, and are found occasionally in Denmark, but are common in hemiboreal-boreal areas of the other Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Scaly Wood Mushroom or Blushing Wood Mushroom. However, Scaly Wood Mushroom and Blushing Wood Mushroom contain several bioactive constituents:

Bioactive constituents

Phenylhydrazine derivatives: Scaly Wood Mushroom and Blushing Wood Mushroom, like other edible mushrooms from the “Rubescentes” group, may contain considerable amounts of agaritine (β -N-[γ -glutamyl]-4-(hydroxymethyl)phenylhydrazine).

According to Schulzová *et al.* (2009) Scaly Wood Mushroom may contain 975 mg agaritine (1 sample) and Blushing Wood Mushroom from non-detectable to 920 mg/kg fresh weight (14 samples, mean content 210 mg per kg fresh weight).

The structural formula of agaritine is shown below:

Agaritine

Agaritine, or at least its metabolites, are indicated to give rise to tumours in mice by a genotoxic mechanism. Therefore, it cannot be excluded that consumption of Scaly Wood Mushroom, Blushing Wood Mushroom and other not yellowing, edible *Agaricus* species containing considerable amounts of agaritine may constitute a cancer risk to humans. (for further information see risk assessment of Button Mushroom, *Agaricus bisporus*).

Blushing Wood Mushroom has been reported to contain N-nitraminoalanine and its decarboxylation product N-nitroethylenediamine (Chilton & Hsu, 1975). The level of these two constituents in the mushroom is not given. Sterner *et al.* (1982) tested the two compounds (prepared by synthesis) for mutagenicity in the Ames test, using the *Salmonella typhimurium* strains TA98, TA100 and TA2637. Only, N-nitroethylenediamine was found mutagenic and only in the strain TA100 in the presence of microsomal enzymes. Sterner *et al.* (1982) also tested a sample of Blushing Wood Mushroom, which was found to be weakly positive in all three *Salmonella* strains. However, Sterner *et al.* (1982) give no information, whether they had controlled for the potential presence of histidine in the extract. It is well known that false positives may be obtained in the presence of histidine. Thus, there is no strong indication

that extracts of Blushing Wood Mushroom are mutagenic. It is remarkable, that Sterner *et al.* (1982) were not able to isolate nitramines from the mushroom using the same procedure as Chilton & Hsu (1975). This apparent discrepancy do they suggest may be due to different chemical races or taxonomic problems.

Cadmium: Scaly Wood Mushroom, Blushing Wood Mushroom and other *Agaricus* species belong to the group of reddening *Agaricus* species (“Rubescentes”) which may become reddish (or brownish), when cut or rubbed. Contrary to the group of yellowing *Agaricus* species (“Flavescentes”), these reddening *Agaricus* species do not bioaccumulate the toxic metal cadmium (Laub *et al.*, 1977; Lodenius *et al.*, 1981; Mowitz, 1980; Seeger, 1978; 1982).

Recommendation

Scaly Wood Mushroom, Blushing Wood Mushroom and other redishing (not yellowing), edible *Agaricus* species:

- Due to the potentially high levels of phenylhydrazine derivatives in redishing (not yellowing) *Agaricus* species, they should not be eaten in larger amounts (see the risk assessment of Button Mushroom (*Agaricus bisporus*)).

Listing 2

Database search information

SciFinder by 1 February 2012. Keywords: *Agaricus langii* or *Agaricus sylvaticus*, *Agaricus* and cadmium, *Agaricus* and agaritine.

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Agaricus species, other yellowing, e.g. *A. essettei* Bon
(*A. abruptibulbus* (Peck) Kauffm. s.auct, *A. sylvicola*
(Vittad.) Peck s. str. and *A. urinascens* (F. H. Møller &
Jul. Schäff.) Singer (*A. excellens* (F. H. Møller)
F. H. Møller, *A. macrosporus* (F. H. Møller & Jul. Schäff.)
Pilát non Montagne)

Agaricus species, other yellowing (DK: Andre gulnende champignoner,
FI: Muut kellertävät *Agaricus* lajin herkkusienet, IS: Aðrar kempur sem
gulna við hnjask, NO: Andre gulnende sjampinjonger, SE: Andra gul-
nande *Agaricus* arter).

A. sylvicola

A. urinascens

Background and risk assessment

Several other yellowing *Agaricus* species ("Flavescentes") than Horse Mushroom (*A. arvensis*) and The Prince (*A. augustus*) are considered edible and collected for consumption, e.g., Wood Mushroom (*A. sylvicola*) and Macro Mushroom (*A. urinascens*).

They are all saprotrophic. Wood Mushroom is growing in forests, scrubs and parks and is common in Denmark and southern parts of Finland, Norway and Sweden where it is also found occasionally in subarctic/subalpine areas. It is rare in Iceland. Macro Mushroom is growing in meadows, steppes, alvar habitats and open places in forests and is occasional in Denmark and southern parts of Finland, Norway and Sweden, but rare in boreal-alpine areas (Knudsen & Vesterholt, 2012).

No intoxications have been reported after consumption of these two or other edible yellowing *Agaricus* species. However, edible yellowing *Agaricus* species contain several bioactive constituents:

Bioactive constituents

Phenylhydrazine derivatives: They all contain high or very high amounts of agaritine (β -N-[γ -glutamyl]-4-(hydroxymethyl)phenylhydrazine).

Wood Mushroom collected in the Czech Republic was found to contain 733–1,940 mg agaritine/kg fresh weight (6 samples, mean content 1,590 mg per kg fresh weight, whereas 7 samples of *A. essetii*⁶ contained 1,740–3,960 (mean content 2,610) mg per kg fresh weight (Schulzová *et al.*, 2009). Stijve and co-workers reported approximately 50–1,200 mg agaritine/kg fresh weight in mushrooms purchased or collected in various European countries and the USA (5 samples, mean content 520 mg per kg fresh weight; Stijve *et al.*, 1986), and between 0.5 and 1.5% of the dried mushroom as being agaritine, corresponding to a level between 500 and 1,500 mg agaritine/kg fresh weight (Stijve & Pittet, 2000). Wood Mushroom also contains another type of phenylhydrazine derivatives, schaefferals, which are hydrazones chemically formed by reaction between 4-(formyl)phenylhydrazine and aromatic aldehydes. The schaefferals were isolated in amounts of about 10 mg per kg fresh mushroom (Kileci-Ksoll *et al.*, 2010).

In Macro Mushroom collected in the Czech Republic, the level of agaritine was 1,050–1,620 mg/kg fresh weight (4 samples, mean content 1,420 mg/kg fresh weight) (Schulzová *et al.*, 2009). Stijve and co-workers reported levels between 30 and 2,500 mg/kg fresh weight in samples collected in various countries (5 samples, mean content 680 mg/kg fresh weight) (Stijve *et al.*, 1986) and a level between 1,500 and 2,500 mg per kg fresh mushroom (Stijve & Pittet, 2000). A structurally related phenylhydrazine derivative, the aldehyde agaritinal (β -N-[γ -glutamyl]-4-(formyl)phenylhydrazine) has also been demonstrated in Macro Mushroom at a level between 500 and 1,500 mg per kg fresh weight (Stijve & Pittet, 2000).

The structural formulae of agaritine, agaritinal and schaefferals are shown below.

⁶ According to Knudsen & Vesterholt (2012) *Agaricus essetii*, is possibly only a large-spored variant of *A. sylvicola* (Wood Mushroom).

Agaritine, or at least its metabolites, are indicated to give rise to tumours in mice by a genotoxic mechanism. Therefore, it cannot be excluded that consumption of yellowing *Agaricus* species which may contain large amounts of agaritine and structurally related phenylhydrazine derivatives may constitute a cancer risk to humans (for further information, see risk assessment of Button Mushroom, *A. bisporus*).

Cadmium: The group of yellowing *Agaricus* species ("Flavescentes"), which have an anise or bitter almond-like smell and become yellowish when rubbed or bruised bioaccumulates the highly toxic metal cadmium. Thus, in general the edible yellowing *Agaricus* species contain much higher cadmium levels than found in other edible mushrooms. In 10 studies, approximately 200 samples from several different edible yellowing *Agaricus* species have been analysed for cadmium. In most cases the levels were between 1 and 10 mg with mean values between 3 and 8 mg cadmium per kg fresh mushroom (Andersen *et al.*, 1982; Laub *et al.*,

1977; Lind *et al.*, 1995; Lodenius *et al.*, 1981; Meisch *et al.*, 1977; Meisch *et al.*, 1979; Mowitz, 1980; Seeger, 1978; Stijve & Besson, 1976; Woggon & Bickerich, 1978).

Cadmium is a highly toxic metal with a very long half-life in humans, ranging from 10 to 30 years. It is primarily toxic to the kidney where it accumulates and may cause renal dysfunction, which may progress to renal failure. It can also cause bone demineralisation. A health based guidance value for cadmium; a Tolerable Weekly Intake (TWI) of 2.5 microgram cadmium per kg body weight has been established by the European Food Safety Authority (EFSA), based on the renal tubular effects (EFSA, 2009; 2011). EFSA estimates that the mean weekly dietary exposure to cadmium in Europe is 2.3 microgram per kg body weight and that regular consumers of wild mushrooms have a higher dietary exposure, around 4.3 microgram per kg body weight per week (EFSA, 2009; 2012). Although the current dietary exposure is unlikely to cause adverse renal effects in European consumers, EFSA concludes there is a need to reduce the exposure to cadmium because of the very small safety margin (EFSA, 2012). Furthermore, WHO's International Agency for Research on Cancer has concluded "There is *sufficient evidence* in humans for the carcinogenicity of cadmium and cadmium compounds" and "There is *sufficient evidence* in experimental animals for the carcinogenicity of cadmium compounds" (IARC, 1993; 2012).

In order to protect public health, the European Commission has set limits for cadmium in a series of food items, including a maximum limit of 1.0 mg per kg fresh weight for mushrooms, except for the most frequently traded mushrooms Button Mushroom (*Agaricus bisporus*), Oyster Mushroom (*Pleurotus ostreatus*) and Shiitake (*Lentinula edodes*) for which a limit of 0.20 mg per kg fresh weight for has been set (EU Commission, 2006).

Recommendation

- As edible yellowing *Agaricus* species efficiently bioaccumulate cadmium, and as the amount in the mushrooms varies considerably, the content of this toxic and carcinogenic metal in yellowing, edible *Agaricus* species needs to be controlled regularly.
- Due to the potentially high levels of phenylhydrazine derivatives and cadmium in the *Agaricus* species, yellowing, edible species of the *Agaricus* genus should not be eaten in larger amounts (regarding phenylhydrazine derivatives, see *Agaricus bisporus* (Button Mushroom) risk assessment).

Listing 2

Database search information

SciFinder by February 2012. Keywords: *Agaricus* and *abruptibulbus* or *essettei* or *excellens* or *silvicola* or *sylvicola* or *urinascens*. PubMed by May 2012. Keywords: *Agaricus* and cadmium. *Agaricus* and agaritine or agaritinal.

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Agaricus sylvaticus Schaeff.

See *Agaricus* species, other not yellowing.

Agaricus sylvicola (Vittad.) Peck s. str.

See *Agaricus* species, other yellowing.

Agaricus urinascens (F. H. Møller & Jul. Schäff.) Singer

See *Agaricus* species, other yellowing.

Albatrellus ovinus (Schaeff.) Kotl. & Pouzar

Forest Lamb (DK: Hvidlig Fåreporesvamp, FI: Lampaankääpä, IS: no Icelandic name, NO: Fåresopp, SE: Fårticka).

Background and risk assessment

Forest Lamb (*Albatrellus ovinus*) is an edible mushroom.

It forms mycorrhiza in coniferous forests, mainly with Spruce (*Picea*). It is very rare or extinct in Denmark, not found in Iceland but common in the other Nordic countries in temperate, hemiboreal and boreal zones (Hansen & Knudsen, 1992).

There are no intoxications reported after consumption of Forest Lamb, and no natural toxicant constituting a risk for humans has been identified in the mushroom. However, Forest Lamb contains some potentially bioactive phenolic compounds.

Bioactive constituents

Triprenylphenolic compounds: Forest Lamb contains a number of related triprenyl(farnesyl) phenolic compounds, either grifolin or neogrifolin derivatives (Besl *et al.*, 1977; Dekermendjian *et al.*, 1997; Nukata *et al.*, 2002; Vrkoc *et al.*, 1977). The structural formulae of two of these phenols, grifolin and scutigeral are shown below.

Grifolin

Scutigeral

Several of these phenols have been demonstrated to have biological activities *in vitro*, e.g. effect on central nervous system receptors, inhibition of various cancer cell lines, antibacterial, antioxidative and anti-inflammatory effects (Dekermendjian *et al.*, 1997; Hellwig *et al.*, 2003; Hirata & Nakanishi, 1950; Jin *et al.*, 2007; Luo *et al.*, 2011a, 2011b; Nukata *et al.*, 2002; Szallazi *et al.*, 1999; Ye *et al.*, 2005; Ye *et al.*, 2007), but they have not been studied *in vivo*.

It is not possible to evaluate whether these *in vitro* bioactive compounds in Forest Lamb may have any effect in humans consuming this mushroom, being it beneficial or adverse effects. Furthermore, the quantitative occurrence of these constituents in the mushroom is generally not well described.

Animal studies: Nieminen and co-workers (2006) have published a study on the potential myo- and hepatotoxic effects of different wild mushrooms in mice. The animals (six females per group) were for 5 days supplied large quantities of dried and powdered wild mushrooms in the feed. One of the mushrooms, Forest Lamb, was given as 1.8%, 3.6% or 5.4% of the feed. The feeding resulted in exposures corresponding to 21, 32 or 59 g per kg body weight of the mice per day, respectively, corresponding to a human exposure of 1.5, 2.5 or 4.2 kg fresh mushroom per person (70 kg) per day, respectively. All mice were healthy at the end of the study. Of the many clinical-chemical parameters studied the plasma LDL cholesterol concentrations were significantly lower at all doses. The plasma creatine kinase activity was significantly increased at the highest dose level. The plasma triacylglycerol concentration was significantly

lower at the highest dose. No histological pathological changes in studied organs were found. Whether the observed increased creatine kinase (indicator of myopathy) in mice eating very high amounts of Forest Lamb may be relevant for humans eating very large amount of the mushroom is difficult to evaluate.

Recommendation

Forest Lamb (*Albatrellus ovinus*) is red-listed as regionally extinct in Denmark. Should only be marketed in the Nordic countries, if it is from countries where it is cultivated or not red-listed.

Listing 1

Database search information

SciFinder by January 2012. PubMed by January 2012. Keywords: *Albatrellus*.

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Amanita caesarea (Scop.) Pers.

Caesar's Amanita (Caesar's Mushroom) (DK: Kejser-Fluesvamp, FI: Keisari-kärpässieni, IS: Keisaraserkur, NO: Keiserfluesopp, SE: (Kejsarflugsvamp).

Background and risk assessment

Caesar's Amanita (*Amanita caesarea*) is a highly appreciated edible mushroom.

It forms mycorrhiza in both deciduous and coniferous woods with Beech (*Fagus*), Oak (*Quercus*), Chestnut (*Castanea*) and Pine (*Pinus*). It is found in Mediterranean to warm temperate areas, but also in Asia and North America. It is not found in the Nordic countries (Gminder & Kriegelsteiner, 2003).

There are no intoxications reported after consumption of Caesar's Amanita, and no natural toxicant constituting a risk for humans has been identified in the mushroom.

Recommendation

No special recommendation.

Listing 1

Database search information

PubMed by June 2012. Keywords: *Amanita caesarea*.

References

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Amanita fulva Fr.

Tawny Grisette (Orange-Brown Ringless Amanita) (DK: Brun Kam-Fluesvamp, FI: Ruostekärpässieni, IS: Rauðserkur, NO: Brun kamfluesopp, SE: Brun kamskivling (Gulbrun kamskivling)).

Background and risk assessment

Tawny Grisette (*Amanita fulva*) is an edible mushroom.

It forms mycorrhiza in deciduous and mixed forest, with Birch (*Betula*), Oak (*Quercus*), and more rarely with Beech (*Fagus*). It is very common in temperate and subalpine areas of the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Tawny Grisette, and no natural toxicant constituting a risk for humans has been identified in the mushroom. There are no intoxications reported after consumption of Tawny Grisette, and no natural toxicant constituting a risk for humans has been identified in the mushroom.

Recommendation

Immature Tawny Grisette (especially when it looks like “small hen’s eggs”) may be mistaken for the deadly poisonous Deathcap (*A. phalloides*) or Destroying Angel (*A. virosa*) which also may look like “small hen’s eggs,” when they are very young (See Chapter 4).

The mushroom is therefore not regarded as suitable for commercial marketing.

Listing 3

Database search information

PubMed by June 2012. Keywords: *Amanita fulva*.

References

Knudsen H & Vesterholt J (2012): Funga Nordica. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. Nordsvamp: 1–1083.

Amanita rubescens Pers.

Blusher (DK: Rødmende Fluesvamp, FI: Ruskokärpässieni, IS: No Icelandic name, NO: Rødnende fluesopp, SE: Rodnande flugsvamp).

Background and risk assessment

Blusher (*Amanita rubescens*) is considered as an edible mushroom.

It forms mycorrhiza with both deciduous and coniferous trees. It is very common in temperate-hemiboreal areas, common in boreal areas and occasional in subarctic-subalpine areas of the Nordic countries (Knudsen & Vesterholt, 2012).

A few reports of severe gastrointestinal symptoms after consumption of Blusher have been described, but it is not clear whether the mushrooms have been sufficiently cooked (Beug *et al.*, 2006). Some bioactive constituents have been reported in Blusher.

Bioactive constituents

Cytolysins: Blusher contains the hemolysin rubescenslysin, which is a potent toxicant after parenteral administration to mice or rats (Seeger *et al.*, 1981). In experimental studies rubescenslysin damages various organs and tissues, isolated from experimental animals, within a few minutes in a dose-dependent and practically irreversible manner, as well as causes lysis of human erythrocytes and leucocytes (Odenthal *et al.*, 1982; Seeger,

1980). However, this cytolytic protein is usually either destroyed during cooking and digestion or not absorbed from the gastrointestinal tract.

Amatoxins/phallotoxins: In a study analysing for the occurrence of amatoxins in various toxic and edible mushrooms, Faulstich & Cochet-Meilhac (1976) reported extremely low levels of amatoxins in Blusher, 10^5 times lower levels than in Deathcap (*Amanita phalloides*) and Destroying Angel (*Amanita virosa*). Two different analytical methods were used in these studies, a radioimmunological assay and an enzyme (RNA polymerase B) inhibition assay. It was concluded that the amounts of amatoxins found in the different edible mushrooms, including Blusher (6,5–7,5 microgram per kg fresh Blusher) in these two studies, are of no toxicological concern in relation to consumption of Blusher (Benjamin, 1995; Faulstich & Cochet-Meilhac, 1976).

The occurrence of trace amounts of amatoxins and/or phallotoxins in Blusher has also been reported by Mullersman & Preston (1982) and by Vargas *et al.* (2011), but could not be confirmed in several other studies. However, the limits of detection in the studies by Clarke *et al.* (2012) and Yocum & Simons (1977) were so high that the levels reported by Faulstich & Cochet-Meilhac (1976) would not have been detected. Using sufficiently sensitive and specific analytical methods, as for example the method developed by Enjalbert *et al.* (1992, 1993), neither Hallen *et al.* (2002) nor Faulstich (as referred by Wieland in 1986) could detect any amatoxin in Blusher. The chemical analytical protocol worked out by Enjalbert (1992) included high-performance liquid chromatography and confirmation by mass spectrometry and was sensitive enough to detect nanogram quantities of amatoxins (and phallotoxins). Enjalbert *et al.* (1993) refuted the findings of trace amounts (nanogram/kg) in Blusher (and other edible mushrooms, e.g. Cep (*Boletus edulis*) and Chanterelle (*Cantharellus cibarius*) due to methodological considerations. The levels found by Faulstich & Cochet-Meilhac (1976), either using a radioimmunoassay (RIA) or an enzyme inhibition assay were at the limits of detection and could be accounted for by contamination. When Faulstich repeated the assay in a different laboratory using new glassware, no toxins were detected in Blusher (Wieland, 1986). Furthermore, the results obtained by bioassays, may have been interfered by other compounds in the mushroom extract (Enjalbert *et al.*, 1993; Wieland, 1986).

Altogether, it is unlikely that Blusher contains even trace amounts of amatoxins.

Recommendation

Blusher (*Amanita rubescens*) resembles Panthercap (*A. pantherina*), which is very poisonous (See Chapter 4).

The mushroom is therefore not regarded as suitable for commercial marketing.

Listing 3

Database search information

PubMed by June 2012. Keywords: *Amanita rubescens*.

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Armillaria borealis Marxm & Korhonen

No English name (DK: Nordlig Honningsvamp (“Honningsvamp”), FI: Pohjanmesisieni, IS: Hunangssveppur, NO: Skoghonningsopp, SE: Vanlig honungsskivling).

Background and risk assessment

There are six species of the genus *Armillaria* (Fr.) Staude (*Armillaria* (P. Karst.) P. Karst.), which can be found in the Nordic countries (Knudsen & Vesterholt, 2012). Five of these have a ring: *A. borealis* and the four very similar *Armillaria* species (“Honey Fungus” species) *A. cepistipes*, *A. lutea* (Bulbous Honey Fungus), *A. mellea* (Honey Fungus) and *A. ostoyae* (Dark Honey Fungus) and these five are often referred to just as “Honey Fungus” (or the “Honey Fungus complex”) as they are very difficult to distinguish from each other.

“Honey Fungus” is generally regarded as edible when thoroughly cooked (Knudsen & Petersen, 2007; Mazzo, 2000; Læssøe, 2004), although it is indicated that even thoroughly cooked “Honey Fungus” sometimes may give rise to intoxications with gastrointestinal disturbances in some individuals (Bresinsky & Besl, 1985; Holmberg & Marklund, 1996; Roth *et al.*, 1990; Seeger, 1995). It is often recommended only to use young caps and possibly also to boil the “Honey Fungus” and then dis-

charge the water before further cooking (Laux, 2001; 2005; Egeland & Myhr, 2003; Korhonen, 1986).

Consumption of insufficiently cooked or raw “Honey Fungus” does give rise to many intoxications (Beug *et al.*, 2006; Muszynska *et al.*, 2011a and Liebenow *et al.*, 1994). The intoxications are relatively harmless, but unpleasant with nausea, diarrhoea and stomach pains (Roth *et al.*, 1990; Watling, 1995). Latency time from consumption to symptoms appearing from 15 minutes to 24 hours has been reported (Roth *et al.*, 1990; Bresinsky & Besl, 1985; Beug *et al.*, 2006). It has been estimated – without specifying the cooking conditions – that 10–20% of people who eat specimens of the *Armillaria mellea* complex do get gastrointestinal disturbances (Benjamin, 1995).

Armillaria species (“Honey Fungus” species) are not listed in the Danish, Norwegian and Swedish guidance lists on edible wild mushrooms.⁷

It grows parasitically or saprotrophically on deciduous and coniferous wood. The occurrence of *A. borealis* is occasional in Denmark, but is very common in boreal zone and occasional in temperate-hemiboreal and sub-alpine zones of the other Nordic countries (Knudsen & Vesterholt, 2012).

There is no information available on whether *A. borealis* is commercially available as dried mushrooms.

Bioactive constituents

The heat-labile toxicant (or toxicants) responsible for intoxication with “Honey Fungus” is unknown, but a series of natural constituents (polysaccharides, sterols, sesquiterpenoids, indole compounds, statins, ergothioneine and others) have been identified in *Armillaria* species (Chen *et al.*, 2012; Lee *et al.*, 2009 ; Guo & Guo, 2011; Momose *et al.*, 2000; Muszynska *et al.*, 2011a; 2011b and Vaz *et al.*, 2011). Several of these compounds have in *in vitro* and animal studies been shown to have significant biological activities (Gao *et al.*, 2009). It is remarkable that approximately 40 sesquiterpene aryl esters (melleolides) have been identified in the *Armillaria mellea* complex (Gao *et al.*, 2009). The majority of these are substituted tricyclic sesquiterpenes with a four-membered, a five-membered and a six-membered ring in which a hydroxyl group is esterified with an aromatic substituted carboxylic acid and often substituted with chlorine in the aromatic or the alicyclic part. Several of these esterified terpenes have experimentally been shown to have antimicrobial and cytotoxic effects (Gao *et al.*, 2009; Shen *et*

⁷ For the time being (June 2012) *Armillaria* species are legally marketed in Finland, but the status is subject to change.

al., 2009; Misiek *et al.*, 2009; Bohnert *et al.*, 2011). It has been suggested that these biologically active esters contribute to the human toxicity of “Honey Fungus” (Teuscher & Lindequist, 1994).

Recommendation

A. borealis (and other Honey Fungus species (*A. cepistipes*, *A. lutea* (Bulbous Honey Fungus), and *A. mellea* (Honey Fungus))) should never be eaten raw, should be thoroughly cooked, and should only be eaten in small amounts, when eaten for the first time.

However, *A. borealis* (as well as *A. cepistipes*, *A. lutea* (Bulbous Honey Fungus) and *A. mellea* (Honey Fungus)) are very difficult to distinguish from the toxic Dark Honey Fungus (*A. ostoyae*), and should therefore not be used for commercial marketing.

Database search information

PubMed June 2012. Keywords: *Armillaria*.

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Armillaria bulbosa (Barla) Velen. s. auct.

See *Armillaria lutea*.

Armillaria cepistipes (Velen) X.L. Mao

No English name (DK: Knoldfodet Honningsvamp (“Honningsvamp”), FI: No Finnish name, IS: Hunangssveppur, NO: Hagehonningsopp, SE: Finfjällig honungsskivling).

Background and risk assessment

There are six species of the genus *Armillaria* (Fr.) Staude (*Armillaria* (P. Karst.) P. Karst.), which can be found in the Nordic countries (Knudsen & Vesterholt, 2012). Five of these have a ring: *A. borealis* and the four very similar *Armillaria* species (“Honey Fungus” species) *A. cepistipes*, *A. lutea* (Bulbous Honey Fungus), *A. mellea* (Honey Fungus) and *A. ostoyae* (Dark Honey Fungus) and these five are often referred to just as “Honey Fungus” (or the “Honey Fungus complex”) as they are very difficult to distinguish from each other.

“Honey Fungus” is generally regarded as edible when thoroughly cooked (Knudsen & Petersen, 2007; Mazzo, 2000; Læssøe, 2004), although it is indicated that even thoroughly cooked “Honey Fungus” sometimes may give rise to intoxications with gastrointestinal disturbances in some individuals (Bresinsky & Besl, 1985; Holmberg & Marklund, 1996; Roth *et al.*, 1990; Seeger, 1995). It is often recommended only to use young caps and possibly

also to boil the “Honey Fungus” and then discharge the water before further cooking (Laux, 2001; 2005; Egeland & Myhr, 2003; Korhonen, 1986).

Consumption of insufficiently cooked or raw “Honey Fungus” does give rise to many intoxications (Beug *et al.*, 2006; Muszynska *et al.*, 2011a and Liebenow *et al.*, 1994). The intoxications are relatively harmless, but unpleasant with nausea, diarrhoea and stomach pains (Roth *et al.*, 1990; Watling, 1995). Latency time from consumption to symptoms appearing from 15 minutes to 24 hours has been reported (Roth *et al.*, 1990; Bresinsky & Besl, 1985; Beug *et al.*, 2006). It has been estimated – without specifying the cooking conditions – that 10–20% of people who eat specimens of the *Armillaria mellea* complex do get gastrointestinal disturbances (Benjamin, 1995).

Armillaria species (“Honey Fungus” species) are not listed in the Danish, Norwegian and Swedish guidance lists on edible wild mushrooms.⁸

It grows parasitically or saprotrophically on deciduous and coniferous wood. The occurrence of *A. cepistipes* is rare in Denmark and in the temperate hemiboreal and boreal zones of the other Nordic countries, but occasional in temperate-hemiboreal and subalpine zone (Knudsen & Vesterholt, 2012).

There is no information available on whether *A. cepistipes* is commercially available as dried mushrooms.

Bioactive constituents

The heat-labile toxicant (or toxicants) responsible for intoxication with “Honey Fungus” is unknown, but a series of natural constituents (polysaccharides, sterols, sesquiterpenoids, indole compounds, statins, ergothioneine and others) have been identified in *Armillaria* species (Chen *et al.*, 2012; Lee *et al.*, 2009; Guo & Guo, 2011; Momose *et al.*, 2000; Muszynska *et al.*, 2011a; 2011b and Vaz *et al.*, 2011). Several of these compounds have in *in vitro* and animal studies been shown to have significant biological activities (Gao *et al.*, 2009). It is remarkable that approximately 40 sesquiterpene aryl esters (melleolides) have been identified in the *Armillaria mellea* complex (Gao *et al.*, 2009). The majority of these are substituted tricyclic sesquiterpenes with a four-membered, a five-membered and a six-membered ring in which a hydroxyl group is esterified with an aromatic substituted carboxylic acid and often substituted with chlorine in the aromatic or the alicyclic part. Several of these

⁸ For the time being (June 2012) *Armillaria* species are legally marketed in Finland, but the status is subject to change.

esterified terpenes have experimentally been shown to have antimicrobial and cytotoxic effects (Gao *et al.*, 2009; Shen *et al.*, 2009; Misiek *et al.*, 2009; Bohnert *et al.*, 2011). It has been suggested that these biologically active esters contribute to the human toxicity of “Honey Fungus” (Teuscher & Lindequist, 1994).

Recommendation

A. cepistipes (and other Honey Fungus species (*A. borealis*, *A. lutea* (Bulbous Honey Fungus), and *A. mellea* (Honey Fungus)) should never be eaten raw, should be thoroughly cooked, and should only be eaten in small amounts, when eaten for the first time.

However, *A. cepistipes* (as well as *A. borealis*, *A. lutea* (Bulbous Honey Fungus) and *A. mellea* (Honey Fungus)) are very difficult to distinguish from the toxic Dark Honey Fungus (*A. ostoyae*), and should therefore not be used for commercial marketing.

Database search information

PubMed June 2012. Keywords: *Armillaria*.

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Armillaria gallica Marxm. & Romagn.

See *Armillaria lutea*.

Armillaria lutea Gillet (*A. gallica* Marxm. & Romagn.,
A. bulbosa (Barla) Velen.s. auct.)

Bulbous Honey Fungus (DK: Køllestokket Honningsvamp (“Honningsvamp”), FI: Nuijamesisieni, IS: Hunangssveppur, NO: Klubbehonningsopp, SE: Klubbhonungsskivling).

Background and risk assessment

There are six species of the genus *Armillaria* (Fr.) Staude (*Armillaria* (P. Karst.) P. Karst.), which can be found in the Nordic countries (Knudsen & Vesterholt, 2012). Five of these have a ring: *A. borealis* and the four very similar *Armillaria* species (“Honey Fungus” species) *A. cepistipes*, *A. lutea* (Bulbous Honey Fungus), *A. mellea* (Honey Fungus) and *A. ostoyae* (Dark Honey Fungus) and these five are often referred to just as “Honey Fungus” (or the “Honey Fungus complex”) as they are very difficult to distinguish from each other.

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Armillaria species (“Honey Fungus” species) are not listed in the Danish, Norwegian and Swedish guidance lists on edible wild mushrooms.⁹

It grows parasitically or saprotrophically on deciduous rarely on coniferous wood. The occurrence of *A. lutea* is very common in Denmark and in temperate, hemiboreal and boreal zones of the other Nordic countries, but less common towards north and rare in Iceland (Knudsen & Vesterholt, 2012).

There is no information available on whether *A. lutea* is commercially available as dried mushrooms.

Bioactive constituents

The heat-labile toxicant (or toxicants) responsible for intoxication with “Honey Fungus” is unknown, but a series of natural constituents (polysaccharides, sterols, sesquiterpenoids, indole compounds, statins, ergothioneine and others) have been identified in *Armillaria* species (Chen *et al.*, 2012; Lee *et al.*, 2009; Guo & Guo, 2011; Momose *et al.*, 2000; Muszynska *et al.*, 2011a; 2011b and Vaz *et al.*, 2011). Several of these compounds have in *in vitro* and animal studies been shown to have significant biological activities (Gao *et al.*, 2009). It is remarkable that approximately 40 sesquiterpene aryl esters (melleolides) have been identified in the *Armillaria mellea* complex (Gao *et al.*, 2009). The majority of these are substituted tricyclic sesquiterpenes with a four-membered, a five-membered and a six-membered ring in which a hydroxyl group is esterified with an aromatic substituted carboxylic acid and often substituted with chlorine in the aromatic or the alicyclic part. Several of these

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esterified terpenes have experimentally been shown to have antimicrobial and cytotoxic effects (Gao *et al.*, 2009; Shen *et al.*, 2009; Misiek *et al.*, 2009; Bohnert *et al.*, 2011). It has been suggested that these biologically active esters contribute to the human toxicity of “Honey Fungus” (Teuscher & Lindequist, 1994).

Recommendation

Bulbous Honey Fungus (*A. lutea*) and other Honey Fungus species (*A. cepistipes*, *A. borealis*, and *A. mellea* (Honey Fungus)) should never be eaten raw, should be thoroughly cooked, and should only be eaten in small amounts, when eaten for the first time,

However, Bulbous Honey Fungus (*A. lutea*, as well as *A. cepistipes*, *A. borealis* and *A. mellea* (Honey Fungus)) are very difficult to distinguish from the toxic Dark Honey Fungus (*A. ostoyae*), and should therefore not be used for commercial marketing.

Database search information

PubMed June 2012. Keywords: *Armillaria*.

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Armillaria mellea (Vahl.) P. Kumm.
(*Armillariella mellea* (Vahl.) P. Karst.)

Honey Fungus (DK: Ægte Honningsvamp (“Honningssvamp”), FI: Keltamesisieni, IS: Hunangssveppur, NO: Ekte honningsopp, SE: Sydlig honungsskivling).

Background and risk assessment

There are six species of the genus *Armillaria* (Fr.) Staude (*Armillaria* (P. Karst.) P. Karst.), which can be found in the Nordic countries (Knudsen & Vesterholt, 2012). Five of these have a ring: *A. borealis* and the four very similar *Armillaria* species (“Honey Fungus” species) *A. cepistipes*, *A. lutea* (Bulbous Honey Fungus), *A. mellea* (Honey Fungus) and *A. ostoyae* (Dark Honey Fungus) and these five are often referred to just as “Honey Fungus” (or the “Honey Fungus complex”) as they are very difficult to distinguish from each other.

“Honey Fungus” is generally regarded as edible when thoroughly cooked (Knudsen & Petersen, 2007; Mazzo, 2000; Læssøe, 2004), although it is indicated that even thoroughly cooked “Honey Fungus” sometimes may give rise to intoxications with gastrointestinal disturbances in some individuals (Bresinsky & Besl, 1985; Holmberg & Marklund, 1996; Roth *et al.*, 1990; Seeger, 1995). It is often recommended only to use young caps and possibly

also to boil the “Honey Fungus” and then discharge the water before further cooking (Laux, 2001; 2005; Egeland & Myhr, 2003; Korhonen, 1986).

Consumption of insufficiently cooked or raw “Honey Fungus” does give rise to many intoxications (Beug *et al.*, 2006; Muszynska *et al.*, 2011a; Liebenow *et al.*, 1994 and Seeger, 1995). The intoxications are relatively harmless, but unpleasant with nausea, diarrhoea and stomach pains (Roth *et al.*, 1990; Watling, 1995). Latency time from consumption to symptoms appearing from 15 minutes to 24 hours has been reported (Roth *et al.*, 1990; Bresinsky & Besl, 1985; Beug *et al.*, 2006). It has been estimated – without specifying the cooking conditions – that 10–20% of people who eat specimens of the *Armillaria mellea* complex do get gastrointestinal disturbances (Benjamin, 1995).

Armillaria species (“Honey Fungus” species) are not listed in the Danish, Norwegian and Swedish guidance lists on edible wild mushrooms.¹⁰

It grows parasitically or saprotrophically on deciduous and coniferous wood. The occurrence of *A. mellea* is occasional in Denmark, very rare in Sweden (Scania), but otherwise not found in the Nordic countries, (Knudsen & Vesterholt, 2012).

There is no information available on whether *A. mellea* is commercially available as dried mushrooms.

Bioactive constituents

The heat-labile toxicant (or toxicants) responsible for intoxication with “Honey Fungus” is unknown, but a series of natural constituents (polysaccharides, sterols, sesquiterpenoids, indole compounds, statins, ergothioneine and others) have been identified in *Armillaria* species (Chen *et al.*, 2012; Lee *et al.*, 2009; Guo & Guo, 2011; Momose *et al.*, 2000; Muszynska *et al.*, 2011a; 2011b and Vaz *et al.*, 2011). Several of these compounds have in *in vitro* and animal studies been shown to have significant biological activities (Gao *et al.*, 2009). It is remarkable that approximately 40 sesquiterpene aryl esters (melleolides) have been identified in the *Armillaria mellea* complex (Gao *et al.*, 2009). The majority of these are substituted tricyclic sesquiterpenes with a four-membered, a five-membered and a six-membered ring in which a hydroxyl group is esterified with an aromatic substituted carboxylic acid and often substituted with chlorine in the aromatic or the alicyclic part. Several of these esterified terpenes have experimentally been shown to have antimicro-

¹⁰ For the time being (June 2012) *Armillaria* species are legally marketed in Finland, but the status is subject to change.

bial and cytotoxic effects (Gao *et al.*, 2009; Shen *et al.*, 2009; Misiek *et al.*, 2009; Bohnert *et al.*, 2011). It has been suggested that these biologically active esters contribute to the human toxicity of “Honey Fungus” (Teuscher & Lindequist, 1994).

Recommendation

A. mellea (Honey Fungus) and other Honey Fungus species (*A. cepistipes*, *A. lutea* (Bulbous Honey Fungus), and *A. borealis*) should never be eaten raw, should be thoroughly cooked, and should only be eaten in small amounts, when eaten for the first time.

However, *A. borealis* (as well as *A. cepistipes*, *A. lutea* (Bulbous Honey Fungus) and *A. mellea* (Honey Fungus)) are very difficult to distinguish from the toxic Dark Honey Fungus (*A. ostoyae*), and should therefore not be used for commercial marketing.

Database search information

PubMed June 2012. Keywords: *Armillaria*.

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Armillaria obscura (Schaeff.) Horak s. auct.

See *Armillaria ostoyae*.

Armillaria ostoyae (Romagn.)
(*Armillariella ostoyae* (Romagn.) Henrink, *A. obscura*
(Schaeff.) Horak s. auct., *A. polymyces* (Gray)
Singer & Clémançon s. auct.)

Dark Honey Fungus (DK: Mørk Honningsvamp, FI: Mäntymesisieni, IS: Hunanssveppur, NO: Mørk honningsopp, SE: Mörkfällig honungsskivling).

Background and risk assessment

There are six species of the genus *Armillaria* (Fr.) Staude (*Armillaria* (P. Karst.) P. Karst.) which can be found in the Nordic countries (Knudsen & Vesterholt, 2012). Five of these have a ring: *A. borealis* and the four very similar *Armillaria* species ("Honey Fungus" species) *A. cepistipes*, *A. lutea* (Bulbous Honey Fungus), *A. mellea* (Honey Fungus) and *A. ostoyae* (Dark Honey Fungus) and these five are often referred to just as "Honey Fungus" (or the "Honey Fungus complex") as they are very difficult to distinguish from each other.

"Honey Fungus" is generally regarded as edible when thoroughly cooked (Knudsen & Petersen, 2007; Mazzo, 2000; Læssøe, 2004), although it is indicated that even thoroughly cooked "Honey Fungus" sometimes may give rise to intoxications with gastrointestinal disturbances in some individuals (Bresinsky & Besl, 1985; Holmberg & Marklund, 1996;

Roth *et al.*, 1990; Seeger, 1995). It is often recommended only to use young caps and possibly also to boil “Honey Fungus” and then discharge the water before further cooking (Laux, 2001; 2005; Egeland & Myhr, 2003; Korhonen, 1986).

Consumption of insufficiently cooked or raw “Honey Fungus” does give rise to many intoxications ((Beug *et al.*, 2006; Muszynska *et al.*, 2011a; Liebenow *et al.*, 1994 and Seeger, 1995). The intoxications are relatively harmless, but unpleasant with nausea, diarrhoea and stomach pains (Roth *et al.*, 1990; Watling, 1995). Latency time from consumption to symptoms appearing from 15 minutes to 24 hours has been reported (Roth *et al.*, 1990; Bresinsky & Besl, 1985; Beug *et al.*, 2006). It has been estimated – without specifying the cooking conditions – that 10–20% of people who eat specimens of the *Armillaria mellea* complex do get gastrointestinal disturbances (Benjamin, 1995).

However, Dark Honey Fungus (*A. ostoyae*) is especially suspected to give rise to intoxications (Knudsen & Vesterholt, 2012), even when cooked (Courtecuisse, 1994; Benjamin, 1995; Lamaison & Polese, 2005).

Armillaria species (“Honey Fungus” species) are not listed in the Danish, Norwegian and Swedish guidance lists on edible wild mushrooms.¹¹

Dark Honey Fungus grows parasitically or saprotrophically on coniferous, less often on deciduous wood. It is common in Denmark and temperate-hemiboreal areas of the other Nordic countries and occasional in boreal-subalpine parts (Knudsen & Vesterholt, 2012).

There is no information available, whether Dark Honey Fungus occasionally is commercially available as dried mushrooms as has been shown to be the case for Honey Fungus (*A. mellea*).

Bioactive constituents

The heat-labile toxicant (or toxicants) responsible for intoxication with “Honey Fungus” is unknown, but a series of natural constituents (polysaccharides, sterols, sesquiterpenoids, indole compounds, statins, ergothioneine and others) have been identified in *Armillaria* species (Chen *et al.*, 2012; Lee *et al.*, 2009; Guo & Guo, 2011; Momose *et al.*, 2000; Muszynska *et al.*, 2011a; 2011b and Vaz *et al.*, 2011). Several of these compounds have in *in vitro* and animal studies been shown to have significant biological activities (Gao *et al.*, 2009).

¹¹ For the time being (June 2012) *Armillaria* species are legally marketed in Finland, but the status is subject to change.

It is remarkable that approximately 40 sesquiterpene aryl esters (melleolides) have been identified in the *Armillaria mellea* complex (Gao *et al.*, 2009). The majority of these are substituted tricyclic sesquiterpenes with a four-membered, a five-membered and a six-membered ring in which a hydroxyl group is esterified with an aromatic substituted carboxylic acid and often substituted with chlorine in the aromatic or the alicyclic part. Several of these esterified terpenes have experimentally been shown to have antimicrobial and cytotoxic effects (Gao *et al.*, 2009; Shen *et al.*, 2009; Misiek *et al.*, 2009; Bohnert *et al.*, 2011). It has been suggested that these biologically active esters contribute to the human toxicity of “Honey Fungus” (Teuscher & Lindequist, 1994).

Recommendation

Dark Honey Fungus (*A. ostoyae*) may give rise to intoxications, even if thoroughly cooked and should therefore not be used in commercial trade.

The toxicant is not known.

Database search information

PubMed June 2012. Keywords: *Armillaria*.

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Armillaria polymyces (Gray) Singer & Clémançon s. auct.

See *Armillaria ostoyae*.

Auricularia auricula (L.) Underw.

See *Auricularia auricular-judae*.

Auricularia auricula-judae (Bull.) J. Schröt.
(*A. auricula* (L.) Underw.)

Jelly Ear (Jew's Ear, Judae's Ear Fungus (DK: Almindelig Judasøre, FI: *Auricularia* –puunkorvat, IS: Eyrasveppir, NO: Judasøre, SE: Judasöra).

Background and risk assessment

Jelly Ear (*Auricularia auricula-judae*)¹² is an edible mushroom.

It is common in Denmark, occasional in western Scania of Sweden, but otherwise not found in the Nordic countries. It is a saprotrophic mushroom growing on especially Elder (*Sambucus nigra*) close to the sea, but sometimes also on other deciduous trees (Hansen & Knudsen, 1997).

There are no intoxications reported after consumption of Jelly Ear, and no natural toxicant constituting a risk for humans has been identified in the mushroom. Except for trace amounts of hydrogen cyanide and except for potential bioactive polysaccharides, there are no publications available dealing with identified bioactive constituents in Jelly Ear. In

¹² Danish Mycological Society (2012) uses *A. auricula-judae* with the combination (Bull.) J.Schröt. [1888], which is older than the combination (Bull.) Wetts. [1897] used by ArtDatabanken (2011) but younger than the combination (Bull.)Quél. [1886] used by Index Fungorum/Species Fungorum (2012).

one study, hydrogen cyanide was identified at a level of 0.11 mg/kg of fresh mushroom (Shindo *et al.*, 1999). Such low levels are of no health concern in relation to consumption of Jelly Ear.

Recommendation

No special recommendation.

Listing 1

Database search information

SciFinder by January 2012. Keywords: *Auricularia*.

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Auricularia polytricha (Mont.) Sacc. and other *Auricularia* species

Ear species (DK: Kinesisk Judasøre og andre Judasøre-arter, FI: *Auricularia* –puunkorvat, IS: Eyrasveppir, aðrir Eyrasveppir, NO: Geléøre-arter, SE: Skogsöra och andra arter inom geléöronsläktet).

Background and risk assessment

*Auricularia polytricha*¹³ and other *Auricularia* species (“Ear species”) are edible mushrooms.

They are saprotrophs and have a worldwide distribution from the temperate to the tropics, growing on living and dead deciduous trees, decayed stumps or logs (Du *et al.*, 2011; Mao, 2000). The mushroom is cultivated, especially in South-East Asia and China, in large amounts.

There are no intoxications reported after consumption of *A. polytricha*, and no natural toxicant constituting a risk for humans has been identified in the mushroom.

¹³ *Auricularia cornea* Ehrenb. is a preferred name instead of *A. polytricha* (Mont.) Sacc. by several authors (e.g., Roberts, 2001). The latter name is preferred by ArtDatabanken (2011) and Index Fungorum/Species Fungorum (2012) and by other authors (e.g., Du *et al.*, 2011).

Besides studies on potential bioactive polysaccharides, there are only a few publications available on bioactive constituents in *A. polytricha*.

Bioactive constituents

Statins: Lo *et al.* (2012) have identified small amounts (16 mg/kg) of lovastatin in dried fruit bodies of *A. polytricha*, corresponding to approximately 2 mg/kg fresh mushroom. For further information on lovastatin, see *Pleurotus ostreatus*.

Ergothioneine: Traces of ergothioneine, 1.4 mg/kg dry weight, have been identified in fruit bodies of *A. polytricha* (Lo *et al.*, 2012).

Lectins: A lectin with a molecular weight of approximately 23 kDa was isolated from *A. polytricha*. Its hemagglutinating activity was demonstrated using human and rabbit erythrocytes (Yagi & Tadera, 1987) but it is not known whether its carbohydrate binding capacity remains after processing of the mushroom.

Mutagens: Extracts prepared from commercial fruit bodies of *Auricularia* sp. were examined for mutagenic activity in a histidine-independent bacterial test system using the *Salmonella typhimurium* strain TM677 as indicator organisms for mutagenic activity. The extract was claimed to be weakly positive in *S. typhimurium* TM677. However, this could be questioned and the authors remark that more information is required to interpret the observation (Grüter *et al.*, 1991).

Recommendation

No special recommendation.

Listing 1

Database search information

SciFinder by December 2012. Keywords: *Auricularia*.

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Boletus aestivalis (Paulet) Fr.)

See *Boletus reticulatus*.

Boletus badius (Fr.) Fr.)

See *Xerocomus badius*.

Boletus edulis Bull.

Penny Bun, Cep (DK: Spiselig Rørhat, Karl Johan (Karl Johan-Rørhat), FI: Herkkutatti, IS: Kóngssveppur (Ætiboldungur), NO: Steinsopp, SE: Stensopp, Karljohan (“Karljohansvamp”).

Background and risk assessment

Cep (*Boletus edulis*) is a very much appreciated edible mushroom. It is traded as fresh, frozen, canned and dried.

It forms mycorrhiza in both deciduous and coniferous forests. It is very common in Denmark and southern parts of Finland, Norway and Sweden, but rare in subalpine-arctic/alpine areas. It has not been found in arctic/alpine parts of Iceland (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Cep (except that in total seven cases of food allergy to Cep are available in the literature, as well as a case of occupational allergic contact dermatitis described by Simeoni *et al.* (2004) from handling Cep). Beyond allergens also other potential bioactive constituents have been reported in Cep.

Bioactive constituents

Allergens: Two cases after inhalation/ingestion of the mushroom and two cases of anaphylactic reaction after consumption of Cep were identified during a 20-year period at the university hospital in Zürich (Torricelli *et al.*, 1997). The clinical manifestation in the cases differed from

oral allergy syndrome with oropharyngeal itching, tingling, and swelling of the lips to asthma and anaphylactic episodes with severe diarrhoea, generalized urticaria, asthma attacks with dizziness, hypotension and tachycardia, leading to hospitalization. Skin prick tests with unprocessed Cep were strongly positive, and in three of the four cases specific IgE could be identified. In a subsequently reported Italian case of food allergy to Cep general itching and diarrhoea appeared 1 hour after mushroom consumption. Serum immunoglobulins from the patient bound to Cep proteins were separated by Roncarolo *et al.*, (1998) using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The immune-blotting identified three bands, two being clear (26 and 39 kDa), and one at approximately 14 kDa (weak). Thus, in this case allergy to Cep was confirmed by specific IgE in the patient serum. Helbling *et al.* (2002a) mention two additional subjects with anaphylaxis following ingestion of *Boletus* sp. Further insight into the allergens of Cep was given by Helbling *et al.* (2002b). They exposed Cep extracts to simulated gastric fluid and separated the protein digest by SDS-PAGE before supplying sera from two Cep allergic patients to the gels. Serum from one of the patients reacted positively with a 75–80 kDa protein assumed to be digestion-resistant.

Ergothioneine: Ergothioneine has been demonstrated in quite high amounts in the fruit bodies of Cep, 0.53 g/kg (fresh weight) (Ey *et al.*, 2007). It has also been found in an amount of 260 mg/kg (dry weight, mean of 3 samples) in the mycelia (Lo *et al.*, 2012). For further information on ergothioneine, see *Lepista nuda*.

Statins: Lo *et al.* (2012) reported a content of 130 mg/kg (dry weight, mean of 3 samples) in the mycelia of Cep. It is not known whether lovastatin also is formed in the fruit bodies. For further information on statins, see *Pleurotus ostreatus*.

Amatoxins: In a study on amatoxins in various toxic and edible mushrooms Faulstich & Cochet-Meilhac (1976) found extremely low levels of amatoxins in Cep, 10^5 – 10^6 times lower levels than in Deathcap (*Amanita phalloides*) and Destroying Angel (*Amanita virosa*). Two different analytical methods were used, a radioimmunological assay and an enzyme (RNA polymerase B) inhibition assay. The amounts of amatoxins found in the different edible mushrooms including Cep (< 0.2–9 microgram per kg fresh mushroom) are of no toxicological concern in relation to consumption of Cep (Faulstich & Cochet-Meilhac, 1976).

Enjalbert *et al.* (1993) refuted the findings of trace amounts (nanogram/kg) in Cep (and other edible mushrooms, e.g. Blusher (*Amanita rubescens*) and Chanterelle (*Cantharellus cibarius*) due to methodological

considerations. The levels found by Faulstich & Cochet-Meilhac (1976), either using a radioimmunoassay (RIA) or an enzyme inhibition assay, were at the limits of detection and could be accounted for by contamination. When Faulstich repeated the assay in a different laboratory using new glassware, no toxins were detected in Cep, or the other edible mushrooms studied by Faulstich and Cochet-Meilhac in 1976 (Wieland, 1986). Furthermore, the results obtained by bioassays, may have been interfered by other compounds in the mushroom extract (Enjalbert *et al.*, 1993; Wieland, 1986).

Altogether, it is unlikely that Cep contains even trace amounts of amatoxins.

Lectins: At least two bioactive lectins have been isolated from Cep (Bovi *et al.*, 2011; Zheng *et al.*, 2007). Generally, lectins are not thermostable and will be destroyed during cooking. One of the lectins in Cep has been examined during heating and its hemagglutinating activity was reduced to 50% at 65 °C and completely abolished above 75 °C (Zheng *et al.*, 2007). The toxicity (gastrointestinal adverse effects) of some poisonous *Boletus* species (*B. satanas* and *B. venenatus*) is probably due to their content of thermostable lectins as shown by e.g. Gachet *et al.* (1996) and by Horibe *et al.* (2010), respectively. Whether the lectins in Cep could give rise to discomfort in some people eating raw or poorly processed Cep is not known.

Mutagens: According to von Wright and colleagues (1982) extracts of Cep are mutagenic in the Ames test. As this test system detects back-mutations from histidine requirement to histidine auxotrophy, presence of histidine in the test solution has the capability of inducing false positives. The publication of von Wright *et al.* (1982) does not mention whether the potential extraction of histidine in the solution tested was controlled for. The presence of histidine could have resulted in false positives. However, also Grüter *et al.* (1991) could demonstrate a weakly mutagenic activity of extracts of Cep using a histidine-independent bacterial system. Whether the mutagens are destroyed during food preparation is unknown.

Sesquiterpenoids: Three hydroxylated botryane sesquiterpenoids have been identified in Cep. One of them, boledulin A, has in *in vitro* studies been investigated for potential health promoting effects and shown moderate cytotoxic effects, but none of these terpenoids have been studied for potentially adverse effects (Feng *et al.*, 2011).

Indole constituents: Cep contains a series of simple biologically active indole compounds (L-tryptophan, 5-hydroxytryptophan, serotonin, melatonin and tryptamine) (Muszynska *et al.*, 2011, 2012). It was demon-

strated that boiling of Cep fruit bodies significantly changed the content of these indoles. 5-Hydroxytryptophan, melatonin and serotonin were completely decomposed, and tryptophan had been formed in amounts of 84 mg /kg (dry weight) corresponding to approximately 8 mg/kg (fresh weight). Some of these constituents are tissue hormones and neurotransmitters and high intakes of tryptophan can damage the nervous system (Stone *et al.*, 2003).

Animal studies: In a feeding study on Yellow Knight (*Tricholoma equestre*) in mice, a reference group receiving 9 g dried, powdered Cep was included. This dose corresponds to 62 g of fresh Cep, or for a person weighing 70 kg to 4.3 kg Cep/day. The animals were sacrificed after 5 days feeding. Body mass, feed and water intake, liver and kidney weights and biochemical parameters were measured. Feed and water intake and creatine kinase activity were significantly increased compared to the control animals receiving a regular mouse feed (Nieminen *et al.*, 2005).

Recommendation

No special recommendation.

Listing 1

Database search information

SciFinder by January 2012. Keywords: *Boletus*. PubMed by January 2012. Keywords: *Boletus*.

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B. erythropus Pers. s. Fries et auct. plur. non Persoon 1796

See *Boletus luridiformis*.

Boletus luridiformis Rostk. (*B. erythropus* Pers. s. Fries et auct. plur. non Persoon 1796)

Scarletina Bolete (Dotted-Stemmed Bolete) (DK: Punktstokket Indigo-Rørhat, FI: Veritatti, IS: Kóngssveppur, Ætiboldungur, NO: Blodrørsopp, SE: Blodsopp).

Background and risk assessment

Scarletina Bolete (*Boletus luridiformis*) is regarded as an edible mushroom in the Nordic countries.

It forms mycorrhiza with both deciduous and coniferous trees. It is common in Denmark and southern parts of Finland, Norway and Sweden, occasional in the hemiboreal zone of the Nordic countries and more rare in the boreal zone (Knudsen & Vesterholt, 2012).

No intoxications have been reported after consumption of Scarletina Bolete. It is probably moderately toxic when eaten raw as indicated in many mushroom textbooks and field guides (e.g. Knudsen & Petersen, 2007; Philips & Jacobsson, 1996; Roth *et al.*, 1990). However, there are no publications available which show that consumption of thoroughly cooked Scarletina Bolete could give rise to discomfort with or without accompanying alcoholic beverages.

Recommendation

Scarletina Bolete resembles Devils Bolete (*Boletus satanas*) and *Boletus legaliae*, which both are poisonous (see Chapter 4).

The mushroom is therefore not regarded as suitable for commercial marketing.

Listing 3**Database search information**

SciFinder by January 2012. Keywords: *Boletus*. PubMed by January 2012. Keywords: *Boletus*.

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Boletus luridus Schaeff.

Lurid Bolete (DK: Netstokket Indigo-Rørhat, FI: Tauriontatti, IS: No Icelandic name, NO: Ildrørsopp, SE: Eldsopp).

Background and risk assessment

Lurid Bolete (*Boletus luridus*) is regarded as an edible mushroom in the Nordic countries.

It forms mycorrhiza in deciduous forests, parks and gardens. It is occasional in Denmark and the most southern parts of Norway and Sweden and rare in the hemiboreal zone of the Nordic countries and boreal zone of Norway (Knudsen & Vesterholt, 2012).

No intoxications have been reported in the Nordic countries after consumption of Lurid Bolete, but several mushroom field guides and toxicity textbooks state that consumption of raw or insufficiently cooked Lurid Bolete may give rise to gastrointestinal distress (e.g. Knudsen & Petersen, 2007; Philips & Jacobsson, 1996; Roth *et al.*, 1990). The thermolabile toxicant(s) is unknown.

Low concentrations of L-(+)muscarine, up to 20 mg/kg dry weight (corresponding to up to 2 mg/kg fresh weight) have been found in Lurid Bolete. Such low amounts (up to 2 mg L-(+)-muscarine per kg fresh weight) is of no toxicological concern in relation to consumption

of Lurid Bolete (Breskinsky & Besl, 1985; Stadelmann *et al.*, 1976). L-(+)-Muscarine is also found in other boletes; in trace amounts in *Boletus calopus* (Stadelmann *et al.*, 1976) and in very high amounts in *Rubinoletus* species (Pauli & Foot, 2005).

L-(+)- Muscarine

Lurid Bolete is sometimes referred to as giving rise to alcohol intolerance after consumption of the mushroom (Benjamin, 1995, Gerhardt, 1997; Holmberg & Marklund, 1996), but only one scientific publication has described this type of Lurid Bolete-disulfiram like reaction (Budmiger & Kocher, 1982). In this case the intoxication involved three adults consuming a dish containing a mixture of Lurid Bolete and Grey Knight (*Tricholoma terreum*) and Umber-Brown Puffball (*Lycoperdon umbrinum*) together with drinking red wine. Some minutes after the consumption all three became unwell with symptoms such as nausea, vomiting, heart palpitation, dizziness, sweating and others. All three mushroom species had been identified by mycological experts.

As no newer reports have confirmed that intake of Lurid Bolete in combination with alcoholic beverages could give rise to such adverse reactions, it has been speculated, whether the adverse effects reported by Budmiger and Kocher (1982) could have been due to consumption of insufficiently cooked Lurid Bolete (Breskinsky & Besl, 1985).

Recommendation

Lurid Bolete resembles Devils Bolete (*Boletus satanas*) and *Boletus legaliae*, which are both poisonous (see Chapter 4).

The mushroom is therefore not regarded as suitable for commercial marketing.

Listing 3

Database search information

SciFinder by January 2012. Keywords: *Boletus*. PubMed by January 2012. Keywords: *Boletus*.

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Boletus pinicola (Vittad.) A. Venturi

See *Boletus pinophilus*.

Boletus pinophilus Pilát & Dermek
(*B. pinicola* (Vittad.) A. Venturi)

Pine Bolete (“Pine Cep”) (DK: Rødbrun Rørhat (“Karl Johan”), FI: Män-
nynherkkutatti, IS: Greniboldungur, NO: Rødbrun Steinsopp, SE: Röd-
brun stensopp).

Background and risk assessment

Pine Bolete (*Boletus pinophilus*) is a very good edible mushroom like the similar Cep.

It grows mycorrhizal with Pine (*Pinus*). It is common in Denmark and the southern parts of Finland, Norway and Sweden and very common in the boreal zone of the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Pine Bolete, and no inherent toxicants have been identified in the mushroom.

Recommendation

No special recommendation.

Listing 1

Database search information

SciFinder by January 2012. Keywords: *Boletus*. PubMed by January 2012. Keywords: *Boletus*.

References

Knudsen H & Vesterholt J (2012): Funga Nordica. Agaricoid, boletoid, clavaroid, cyphelloid and gastroid genera. Nordsvamp: 1–1083.

Boletus reticulatus Schaeff. (*B. aestivalis* (Paulet) Fr.)

Summer Bolete, “Summer Cep” (DK: Sommer-Rørhat (“Karl Johan”), FI: Tammenherkkutatti, IS: No Icelandic name, NO: Bleklodden steinsopp, SE: Finluden stensopp).

Background and risk assessment

Summer Bolete (*Boletus reticulatus*) is a very good, edible mushroom, like the similar Cep.

It grows mycorrhizal with different deciduous trees. It is common in Denmark and in the southern (temperate) parts of Norway and Sweden. It is occasional in the hemiboreal zone of Finland, Norway and Sweden. Otherwise, it is rare in the Nordic countries (Knudsen & Vesterholt, 2012).

No intoxications have been reported after consumption of Summer Bolete and no inherent toxicants have been identified.

Two lectin systems have been isolated from Summer Bolete: lectin system A, composed of 4 isolectins and lectin system B composed of lectins B1 and B2 (Kozmik & Entlicher, 1997). Whether these lectins, in some individuals, could give rise to discomfort after consumption of raw Summer Bolete is not known.

Recommendation

No special recommendation.

Listing 1

Database search information

SciFinder by January 2012. Keywords: *Boletus*. PubMed by January 2012. Keywords: *Boletus*.

References

- Knudsen H & Vesterholt J (2012): Funga Nordica. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. Nordsvamp: 1–1083.
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Calocybe gambosa (Fr.) Donk (*Lyophyllum gambosum* (Fr.) Singer, *Tricholoma gambosum* (Fr.) P. Kumm., *Tricholoma georgii* (L.) Quél.)

St. George's Mushroom (DK: Vårmusseron, FI: Kevätkaunolakki, IS: Vorkolla, NO: Vårmusserong, SE: Vårmusseron).

Background and risk assessment

St. George's Mushroom (*Calocybe gambosa*) is an appreciated, edible mushroom.

It is a saprotrophic mushroom, growing in fairy rings on rich soil in meadows, pastures, gardens and forests in spring, rarely in autumn. It is common in temperate and occasional in hemiboreal and southern boreal areas of the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of St. George's Mushroom, and no natural toxicants constituting a risk for humans have been identified in the mushroom.

Recommendation

No special recommendation.

Listing 2

Database search information

SciFinder by September 2012. Keywords: *Calocybe*.

References

Knudsen H & Vesterholt J (2012): Funga Nordica. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. Nordsvamp: 1–1083.

Camarophyllus pratensis (Fr.) P. Kumm.

See *Hygrocybe pratensis*.

Cantharellus cibarius Fr.

Chanterelle (“Girolle”) (DK: Almindelig Kantarel (“Kantarel”), FI: Keltavahvero, IS: Kantarella, NO: Kantarell, SE: Kantarell).

Background and risk assessment

Chanterelle (*Cantharellus cibarius*) is edible and much appreciated.

It is a common wild mushroom, which is found in poor coniferous or deciduous forests in the Nordic countries, except in Iceland, where it is occasional (Hansen & Knudsen, 1997). It is mostly sold fresh, but may also be sold as dried or canned.

There are no intoxications reported after consumption of Chanterelle. However, some bioactive constituents have been identified in the mushroom:

Bioactive constituents

Polyunsaturated long-chain fatty acids: When injured Chanterelle may form cibaric acid ((9Z, 13Z, 15E)-14,18-dihydroxy-12-keto-9,13, 15 octadecatrienoic acid) in amounts of up to 100 mg per kg fresh weight (Anke *et al.*, 1996; Pang & Sterner, 1991, Pang *et al.*, 1992). Cibaric acid is not found in the undamaged Chanterelle. It is anticipated to be formed enzymatically from 14,15-dehydrocrepenyic acid, one of the major fatty acids in the fresh Chanterelle. The structural formulae for cibaric acid and 14,15-dehydroxycrepenyic acid are shown below.

Cibaric acid

14,15-Dehydrocrepenylic acid

As cibaric acid is an α , β -unsaturated ketone and potentially may form a beta-diketone by β -diketone-enol tautomerism (EFSA, 2004; 2007), cibaric acid could be suspected to be a mutagen. However, it is negative in *Salmonella typhimurium* tester strains TA98 and TA100 with and without activation with S-9 mix (Anke *et al.*, 1996). Cibaric acid shows very weak antimicrobial and cytotoxic activity and only in five of seventeen bacteria, fungi or cell lines tested (Anke *et al.*, 1996). Cibaric acid has also been shown to have a weak nematocidal activity (Stadler *et al.*, 1994).

As cibaric acid is destroyed by heating at 100 °C for 10 minutes in water, cibaric acid is not of concern with respect to consumption of cooked Chanterelle (Anke *et al.* 1996). Recently, trace amounts of two acetylenic C-18 carboxylic acids have been isolated from Chanterelle: (10E,14Z)-9-oxooctadeca-10,14-dien-12-ynoic acid and the corresponding 9-hydroxy compound. From 9 kg dried fruit bodies were isolated 10.4 and 35.5 mg of the two acids, respectively (Hong *et al.*, 2012). There are no data on heat-stability and the potential toxicity of these two constituents.

Amatoxins: In a study analysing for the occurrence of amatoxins in various toxic and edible mushrooms, Faulstich & Cochet-Meilhac (1976) reported extremely low levels of amatoxins in Chanterelle, 10^5 times lower levels than in Deathcap (*Amanita phalloides*) and Destroying Angel (*Amanita virosa*). Two different analytical methods were used in these studies, a radioimmunological assay and an enzyme (RNA polymerase B) inhibition assay. It was concluded that the amounts of amatoxins found in the different edible mushrooms, including Chanterelle (4.8–5.3 microgram per kg fresh Chanterelle) in these two studies, are of no toxicological concern in relation to consumption of Chanterelle (Benjamin, 1995; Faulstich & Cochet-Meilhac, 1976).

Enjalbert *et al.* (1993) refuted the findings of trace amounts (microgram/kg) in Chanterelle (and other edible mushrooms, e.g. Cep (*Bole-*

tus edulis) and Blusher (*Amanita rubescens*) due to methodological considerations. The levels found by Faulstich & Cochet-Meilhac (1976), either using a radioimmunoassay (RIA) or an enzyme inhibition assay were at the limits of detection and could be accounted for by contamination. When Faulstich repeated the assay in a different laboratory using new glassware, no amatoxins were detected in Chanterelle or the other edible mushrooms studied by Faulstich & Cochet-Meilhac in 1976 (Wieland, 1986). Furthermore, the results obtained by bioassays, may have been interfered by other compounds in the mushroom extract (Enjalbert *et al.*, 1993; Wieland, 1986).

Alltogether, it is unlikely that Chanterelle contains even trace amounts of amatoxins.

Ergothioneine: Lee *et al.* (2009) found 4.1 g/kg of ergothioneine in dried fruit bodies of Chanterelle, corresponding to approximately 400 mg/kg (fresh weight), whereas Ey *et al.* (2007) only found traces, less than 1 mg/kg (fresh weight).

Indole compounds: Chanterelle contains a series of simple biologically active indole compounds (5-hydroxytryptophan, serotonin and tryptamine), but only serotonin in significant amounts, approximately 30 mg/kg (fresh weight) (Muszynska *et al.*, 2011, 2012). It was demonstrated that boiling of Chanterelle fruit bodies significantly changed the content of these indoles. 5-Hydroxytryptophan and serotonin were completely decomposed and tryptophan, tryptamine, melatonin and 5-methyltryptophan had been formed in amounts between 33 and 49 mg/kg (dry weight) corresponding to approximately 3–5 mg/kg of these indoles (fresh weight). Some of these constituents are tissue hormones and neurotransmitters and high intakes of tryptophan can damage the nervous system (Stone *et al.*, 2003).

Mutagens: Grüter *et al.* (1991) has shown that freeze-dried extract of Chanterelle is weakly mutagenic in a histidine-independed bacterial test system. The mutagenic activity was enhanced by metabolic activation. It is not known, if the mutagens are destroyed during cooking of the mushroom.

Animal studies: Nieminen and co-workers (2006) have published a study on the potential myo- and hepatotoxic effects of different wild mushrooms in mice. The animals (six females per group) were for 5 days supplied large quantities of dried and powdered wild mushrooms in the feed. One of the mushrooms, Chanterelle, was given as 1.8%, 3.6% or 5.4% of the feed. The feeding resulted in exposures corresponding to 22, 42 or 64 g per kg body weight of the mice per day, respectively or corresponding to 1.5, 2.9 or 4.5 kg mushroom per person (70 kg) per day of fresh mushroom, respectively. All mice were healthy at the end of the

study. Of the many clinical-chemical parameters studied the plasma creatine kinase activity was significantly increased at the lowest and highest dose level. No histological pathological changes in studied organs were found. Whether the observed (but not dose-related) increased creatine kinase (indicator of myopathy) in mice, eating very high amounts of Chanterelle, may be relevant for humans, eating very large amounts of the mushroom, is difficult to evaluate.

Recommendation

No special recommendations.

Database search information

SciFinder by December 2012. Keywords: *Cantharellus*, *Craterellus*.

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Cantharellus cornucopioides (L.) Fr.

See *Craterellus cornucopioides*.

Cantharellus lutescens Fr.

See *Craterellus lutescens*.

Cantharellus pallens Pilát

No English name (DK: Bleg Kantarel, ("Kantarel"), FI: Kalvasvahvero, IS: No Icelandic name, NO: Blek kantarell, SE: Blek kantarell).

Background and risk assessment

*Cantharellus pallens*¹⁴ is edible and much appreciated.

It is common in Denmark and southern Sweden, occasional in Norway. It is found in Finland, but the frequency is unknown (Hansen & Knudsen, 1997). It is found in rich deciduous forests. It is occasionally sold fresh, dried or canned like the very similar looking Chanterelle (*C. cibarius*).

There are no intoxications reported after consumption of *Cantharellus pallens*.

Due to the close relationship to Chanterelle and, therefore, anticipated compositional similarity, it is possible that *Cantharellus pallens* by injury may form cibacic acid ((9Z, 13Z, 15E)-14,18-dihydroxy-12-keto-9,13, 15 octadecatrienoic acid). This compound has been shown to be formed in

¹⁴ Contrary to the Nordic mycological societies, Index Fungorum/Species Fungorum (2012) does not consider *Cantharellus pallens* Pilát as a separate species, but as a synonym for *C. cibarius* Fr.

injured Chanterelle (Anke *et al.*, 1996; Pang & Steemer, 1991, Pang *et al.*, 1992). On damage of the mushroom it is anticipated to be formed enzymatically from 14, 15-dehydrocrepenyic acid, which is one of the major fatty acids in the fresh *Cantharellus pallens*. For further information on the biological activity of cibaric acid, see the monograph on Chanterelle (*Cantharellus cibarius*). As cibaric acid is destroyed by heating at 100 °C for 10 minutes in water (Anke *et al.* 1996), cibaric acid is not of concern with respect to consumption of cooked *Cantharellus pallens*, should it occur also in this mushroom species.

Recommendation

No special recommendations.

Database search information

SciFinder by December 2012. Keywords: *Cantharellus*, *Craterellus*.

Listing 1

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Cantharellus tubaeformis Fr.

See *Craterellus tubaeformis*.

Chlorophyllum olivieri (Barla) Wellinga (*Lepiota olivieri* Barla, *Macrolepiota olivieri* (Barla) Wasser)

No UK name (DK: Almindelig Rabarberhat ("Rabarber-Parasolhat"), FI: No Finnish name IS: Garðskermill, NO: Maurtueparasollsopp, SE: No Swedish name).

Background and risk assessment

Chlorophyllum olivieri is generally regarded as an edible mushroom (e.g., Knudsen & Vesterholt, 2012). However, it should be thoroughly cooked before consumption (Lohmeyer & Künkele, 2006; Roth *et al.*, 1990).

Some textbooks on mushrooms indicate that Shaggy Parasol may give rise to gastrointestinal distress in some people (Philips, 2006; Ryman & Holmåsén, 1996) or at least that some garden forms or varieties cause gastrointestinal upsets (Bresinski & Besl, 1985; Holmberg & Marklund, 1996; Krikorev, 2007; Lamaison & Poelese, 2004; Læssøe, 2004). Beug *et al.* (2006) have reported 15 cases in the USA in the period 1976–2005 with mild to severe gastrointestinal symptoms and an onset time of 0.1–10 hours (average 4 hours).

It has more recently been established that the mushroom traditionally named Shaggy Parasol, with the scientific name *Macrolepiota rachodes*/*M. rhacodes* (*Lepiota rachodes*/*L. rhacodes*), comprises three very similar species belonging to the genus *Chlorophyllum*. These species are

C. brunneum, *C. olivieri* and *C. rachodes* (Knudsen & Vesterholt, 2012; Lange & Vellinga, 2004). Besides *C. olivieri*, also *C. rachodes* is regarded as edible, whereas *C. brunneum* is considered to be poisonous (Knudsen & Vesterholt, 2012; Lange & Vellinga, 2004). Therefore many earlier reported intoxications after consumption of “Shaggy Parasol” may be due to *C. brunneum*. The English name Shaggy Parasol now covers only *C. rachodes* (British Mycological Society, 2012), but not *C. olivieri* or *C. brunneum*.

C. olivieri grows in coniferous woods, in old ant-hills or litter beds, often forming fairy rings. It is common in temperate hemiboreal and boreal zones, but very rare in subalpine/subarctic zones of the Nordic countries. In Iceland, it is only found in gardens (Knudsen & Vesterholt, 2012; Lange & Vellinga, 2004).

No natural toxicant constituting a risk for humans has been identified in the mushroom.

In 1982, Sterner *et al.* reported that a *Macrolepiota rachodes* (“Shaggy Parasol”) extract was weakly mutagenic as detected by the histidine-requiring *Salmonella typhimurium* strains TA98, TA100 and TA2637 in the Ames assay. The extract was positive in all three strains but the mutagenicity was not enhanced when including a metabolizing system (S9 mix). Sterner *et al.* (1982) gave no information, whether they controlled for the potential presence of histidine in the mushroom extract tested. The presence of histidine could have resulted in false positives. A further uncertainty might be that the mushroom studied could be any of the three *Chlorophyllum* species mentioned.

Recommendation

Chlorophyllum olivieri resembles *Chlorophyllum brunneum*, which is suspected to be poisonous (see Chapter 4). The mushroom is therefore not regarded as suitable for commercial marketing.

Listing 3

Database search information

SciFinder by January 2012. Keywords: *Chlorophyllum* or *Lepiota* or *Macrolepiota*. PubMed by December 2012. Keywords: *Chlorophyllum* or *Lepiota* or *Macrolepiota*.

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Chlorophyllum rachodes (Vittad) Wellinga
(*Macrolepiota rachodes* (Vittad.) Singer,
Lepiota rhacodes (Vittad.) Quél.)

Shaggy Parasol (DK: Ægte Rabarberhat ("Rabarber-Parasolhat"),
FI: Akansieni, IS: No Icelandic name, NO: Rødnende parasollsopp,
SE: Rodnande fjällskivling).

Background and risk assessment

Shaggy Parasol (*Chlorophyllum rachodes*) is generally regarded as an edible mushroom (e.g., Knudsen & Vesterholt, 2012). However, it should be thoroughly cooked before consumption (Lohmeyer & Künkele, 2006; Roth *et al.*, 1990).

Some textbooks on mushrooms indicate that Shaggy Parasol may give rise to gastrointestinal distress in some people (Philips, 2006; Ryman & Holmåsén, 1996) or at least that some garden forms or varieties cause gastrointestinal upsets (Bresinski & Besl, 1985; Holmberg & Marklund, 1996; Krikorev, 2007; Lamaison & Poelese, 2004; Læssøe, 2004). Beug *et al.* (2006) have reported 15 cases in the USA in the period 1976–2005 with mild to severe gastrointestinal symptoms and an onset time of 0.1–10 hours (average 4 hours).

It has more recently been established that the mushroom traditionally named Shaggy Parasol with the scientific name *Macrolepiota rachodes*/*M. rhacodes* (*Lepiota rachodes*/*L. rhacodes*) comprises three very similar species belonging to the genus *Chlorophyllum*. These species are *C. brunneum*, *C. olivieri* and *C. rachodes* (Knudsen & Vesterholt, 2012; Lange & Vellinga, 2004). Besides *C. rachodes* (Shaggy Parasol) also *C. olivieri* are regarded as edible, whereas *C. brunneum* is considered to be poisonous (Knudsen & Vesterholt, 2012; Lange & Vellinga, 2004). Therefore many earlier reported intoxications after consumption of “Shaggy Parasol” may be due to *C. brunneum*. The English name Shaggy Parasol now covers only *C. rachodes* (British Mycological Society, 2012), but not *C. olivieri* or *C. brunneum*.

Shaggy Parasol (*C. rachodes*) grows in deciduous woods, shrubs, parks and gardens, more rarely in coniferous woods, often forming fairy rings. It is occasional or common in temperate and hemiboreal areas of the Nordic countries (Knudsen & Vesterholt, 2012).

No natural toxicant constituting a risk for humans has been identified in the mushroom.

In 1982, Sterner *et al.* reported that a *Macrolepiota rachodes* (“Shaggy Parasol”) extract was weakly mutagenic as detected by the histidine-requiring *Salmonella typhimurium* strains TA98, TA100 and TA2637 in the Ames assay. The extract was positive in all three strains but the mutagenicity was not enhanced when including a metabolizing system (S9 mix). Sterner *et al.* (1982) gave no information, whether they controlled for the potential presence of histidine in the mushroom extract tested. The presence of histidine could have resulted in false positives. A further uncertainty might be that the mushroom studied could be any of the three *Chlorophyllum* species mentioned.

Recommendation

Shaggy Parasol resembles *Chlorophyllum brunneum*, which is suspected to be poisonous (see Chapter 4).

The mushroom is therefore not regarded as suitable for commercial marketing.

Listing 3

Database search information

SciFinder by January 2012. Keywords: *Chlorophyllum* or *Lepiota* or *Macrolepiota*. PubMed by December 2012. Keywords: *Chlorophyllum* or *Lepiota* or *Macrolepiota*.

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Clitocybe connata (Schumach.) Gillet
(*Lyophyllum connatum* (Schumach.) Singer)

White Domecap (DK: Knippe-Gråblad, FI: Nurmitupaskynsikäs, IS: Gráspyrða (Gráknipplingur), NO: Hvit knippesopp, SE: Vit tuvskivling).

Background and risk assessment

White Domecap (*Clitocybe connata*) is no longer considered as an edible mushroom.

It grows on soil in deciduous forests, parks and ruderal areas. It is common in Denmark and in large parts of Finland, Norway and Sweden but occasional in subarctic/subalpine areas (Knudsen & Vesterholt, 2012).

White Domecap was earlier considered edible (e.g., Korhonen, 1976; Lange & Lange, 1970; Ryman & Holmåsen, 1986) and it still is in some more recent textbooks on mushrooms (Mazza, 2000; Nylén, 2012; Philips & Jacobsson, 1996). There are no intoxications reported after consumption of White Domecap. However, some bioactive constituents with potential toxicity have been isolated from the mushroom.

Bioactive constituents

Azoxy compounds: Fugmann & Steglich (1984) have isolated an azoxy compound, lyophyllin, occurring in significant amounts, more than 400 mg per kg, in the fresh mushroom. More recently the presence of lyophyllin in White Domecap was confirmed by Ye *et al.* (1997), who showed that it is formed from N-hydroxy-N',N'-dimethylurea and N-methylhydroxylamine in the mushroom. Lyophyllin is suspected to be mutagenic and carcinogenic due to its similarity to other aliphatic azoxy compounds such as elaiomycin, which is carcinogenic in rats (Schoental, 1969), and methylazoxymethanol, the aglycone of the two toxic cycad glycosides cycasin and macrozamin, which are mutagenic both *in vitro* and *in vivo* and are known to be carcinogenic in experimental animals and suspected to be so also in humans (Hoffmann & Morgan, 1984; Toth, 2000; IARC, 1976; 1987).

Hydroxyurea derivatives: Fugmann & Steglich (1984) also isolated another potentially bioactive constituent, N-hydroxy-N',N'-dimethylurea from the mushroom. The levels were in the range 100–200 mg per kg (fresh weight). The presence of this constituent has been confirmed (Kimura *et al.*, 2005). Like the DNA inhibitor hydroxyurea (hydroxycarbamate), N- or N'-monomethylated hydroxyurea have shown antimitotic effects on female mice vaginal mucosa (Yu & van Scott, 1974). It is not known whether the N', N'-dimethylated hydroxyurea also has antimitotic effects *in vivo*.

Other bioactive constituents

In addition to the suspected toxicants already described in White Domecap, the mushroom contains more than 2 g per kg fresh weight of the chromogen connatin (Fugmann & Steglich, 1984) and unknown quantities of 2-(3,4-dihydroxyphenyl)glycine (Keller *et al.*, 2002). Keller *et al.* (2002) observed that methanol extracts of the mushroom fruit bodies exhibit both radical scavenging and anti-oxidant activity in *in vitro* systems. Kimura *et al.* (2005) confirmed the occurrence of connatin in the mushroom, and in addition noted that the mushroom contains traces of ergothioneine and β -hydroxyergothioneine (approximately 30 mg/kg, fresh weight), both compounds with radical scavenging activity. For further information on ergothioneine, see *Lepista nuda*.

The structural formulae of the bioactive constituents in White Domecap, lyophyllin, N-hydroxy-N',N'- dimethylurea, connatin and β -hydroxyergothioneine, are shown below.

Connatin

β -Hydroxyergothioneine

N-Hydroxy-N',N'-dimethylurea

Lyophyllin

Recommendation

Heat-stable constituents in White Domecap are suspected to be genotoxic and carcinogenic.

Listing 4

Database search information

SciFinder by December 2012. Keywords: *Lyophyllum*. PubMed by December 2012. Keywords: *Lyophyllum* or *Clitocybe connata*.

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Clitocybe nebularis (Batsch) P. Kumm.
(*Lepista nebularis* (Batsch) Harmaja)

Clouded Funnel (DK: Tåge-Tragthat, FI: Härmämalikka, IS: -trektla, NO: Puddertraktsopp, SE: Pudrad trattskevling).

Background and risk assessment

It is questionable whether Clouded Funnel (*Clitocybe nebularis*) can be considered an edible mushroom. It is poisonous when eaten raw (Bresinsky & Besl, 1985; Gerhardt, 1997; Roth *et al.*, 1990) and it does sometimes give rise to intoxications with gastrointestinal distress, even after thorough cooking (Alder, 1960; Knudsen & Petersen, 2007; Lamaison & Polese, 2005; Laux, 2001; Lohmeyer & Künkele, 2006; Læssøe, 2004; Philips, 2006).

Clouded Funnel is a saprotrophic mushroom growing on soil in deciduous and coniferous woods, parks, gardens and ruderal areas, often in fairy rings. It is very common in temperate and hemiboreal zones, and common in boreal zones of the Nordic countries (Knudsen & Vesterholt, 2012).

The toxic constituents in Clouded Funnel are not known. However, several biologically active compounds have been identified in the mushroom.

Bioactive constituents

Nebularine: Already in 1946 Ehrenberg and co-workers isolated nebularine as the active antibiotic compound in the press juice of Clouded Funnel. Nebularine was subsequently identified as 9- β -D-ribofuranosyl-9H-purine (Löfgren & Lünig, 1953; Löfgren *et al.*, 1954), which was confirmed by chemical synthesis of the compound (Brown and Weliky 1953). Its structure makes it likely to be an adenosine analogue. The level of nebularine in the fruit body was small, around 0.002% (Löfgren *et al.*, 1954). Its biosynthesis has been studied in mycelial cultures of the mushroom, which showed that nebularine is produced by a specific enzymatic activity using adenosine as substrate (Brown & Konuk, 1995). Nebularine was shown to have selective antibiotic activity against pathogenic and non-pathogenic bacteria (Ehrenberg *et al.*, 1946; Löfgren and Lünig, 1953) and micro-fungi (Kim *et al.*, 2008), and to be cytotoxic to cultivated mammalian cells (Biesele *et al.*, 1955). Germination of seeds of various plant species in the presence of 0.013% (w/v) aqueous solutions of nebularine resulted in severe inhibition of growth and appearance of chromosomal abnormalities in the root tip cells. Seedlings died after a few days (Milton *et al.*, 1992). Nebularine has also been demonstrated to be toxic to rodents (Brown and Weliky, 1953; Philips *et al.*, 1954).

It seems as nebularine is taken up by cells and phosphorylated, possibly by the enzyme adenosine kinase (Bohr, 1978; Divekar *et al.*, 1972; Lindberg *et al.*, 1967; Schnebli *et al.*, 1967). The triphosphate of nebularine was shown not to be a substrate for the *Escherichia coli* RNA polymerase, but it acts as a competitive inhibitor with ATP for this enzyme, possibly by binding to the polymerase (Bohr, 1978). Further studies by the same investigator showed that nebularine strongly inhibits RNA and DNA synthesis in cancer ascites cells. The most potent inhibition was on the ribosomal components.

Gordon and Brown (1956) studied the metabolism of 9- β -D-ribofuranocyl-9H-purine in Sherman rats. Four hours after intraperitoneal injection of the compound at least three nucleotide-containing purines could be detected in the liver (possibly phosphorylated compounds). No appreciable oxidation to carbon dioxide was noted. A portion is converted to adenine and guanine. It is extensively catalysed to urinary allantoin.

It is not known, whether the small quantities of nebularine, approximately 20 mg/kg (fresh weight) in Clouded Funnel could give rise to adverse effects after consumption of the mushroom. The structural formula of nebularine is shown below.

Nebularine

Lectins: Zaremba (1973) partially purified a substance from an extract of fruit bodies of Clouded Funnel and showed that it agglutinated erythrocytes from several animal species. This early publication gave no data on molecular mass, purity or stability. Subsequently, Horejsi *et al.* (1978) isolated a lectin from fruit bodies of Clouded Funnel, the hemagglutinating action of which was inhibited by N-acetylgalactosamine. It was estimated to consist of two subunits with molecular masses of 19 and 14.5 kDa, but otherwise few details were given. More recently, comprehensive studies have been performed by Pohleven and co-workers. These investigators isolated and characterized a lectin from the fruit bodies of Clouded Funnel. The lectin is a homodimeric protein with each subunit having a molecular mass of 15.9 kDa (Pohleven *et al.*, 2009). It agglutinates bovine and human erythrocytes, in particular blood group A erythrocytes and binding requires the unique glycan N,N'-diacetyllactosediamine. The mechanism of action was deduced and revealed bivalent carbohydrate binding for biological activity (Pohleven *et al.*, 2012). Each monomer of the homodimeric protein has a carbohydrate-binding domain. Due to the similarity of the amino acid sequence of the *C. nebularis* lectin with the ricin B-chain, the lectin was characterized as a ricin B-like lectin. The lectin showed selective antiproliferative effects on human cells cultured *in vitro*; some types of cells were insensitive, others, like leukemic T cells, were sensitive. The lectin was partially resistant to heat-treatment. It had unchanged hemagglutinating activity after 30 minutes of heating up to 50 °C and kept 14% of the activity after 30 minutes at 100 °C. Svaiger *et al.* (2011) demonstrated that the ricin B-like lectin from Clouded Funnel has immunostimulatory effects, inducing maturation and activation of human dendritic cell via the toll-like receptor 4 pathway. The increased understanding on the mechanism of lectin-binding allowed Pohleven *et al.* (2011) to isolate five additional agglutinating lectins from the Clouded Funnel, these being characterized by glucose-, galactose-, sucrose-, lactose-, and sepharose-binding and molecular weights in the region 15–31 kDa. Individual lectins were demonstrated to have insecticidal activity against Fruit Fly (*Drosophila melanogastere*), with the activity descending in

the order sucrose-binding lectin, N-acetylgalactosamine-binding lectin, lactose-sepharose-binding lectin and galactose-sepharose-binding lectin (the other having no activity), and the N-acetylgalactosamine-binding lectin showing anti-nutritional effect on the larvae from Colorado Potatoe Beetle (*Leptinotarsa decemlineata*).

There were no data on the thermostability of the five lectins. It is known from lectins of plants that certain lectins, especially the thermostable ones, may give rise to gastrointestinal discomfort (Liener, 1983).

Protease inhibitors: Brzin *et al.* (2000) isolated a cysteine proteinase inhibitor with an apparent molecular mass of 34 kDa from fruit bodies of Clouded Funnel and called the proteinase inhibitor clitocypin. The clitocypin expression appears to be uniform throughout the fruiting body (Sabotič *et al.*, 2011). The inhibitor was characterized as a homodimer of two 17 kDa units. The 150 amino acid long molecule did not contain any cysteine or methionine residues and accordingly no inter-subunit disulfide bonds and also no carbohydrate linkage. The monomer sequence showed no similarity to any other known cysteine proteinase inhibitor but had some similarity to a lectin-like family of proteins specifically found in mushrooms. It demonstrated tight binding to proteins such as papain, cathepsin B and bromelain but was inactive towards cathepsin H, trypsin and pepsin. The binding of the clitocypin with papain-like cysteine proteases has been studied in crystalized material (Renko *et al.*, 2010). By inhibiting protein degradation, proteinase inhibitors have anti-nutritional effects. Proteinase inhibitors are frequently destroyed by processing but the clitocypin retained its activity and dimer composition after boiling in water.

Sesquiterpenoids: Four sesquiterpenoids, two named nebularic acid A and B and two named nebularic lactones A and B, were isolated from a cultured strain of Clouded Funnel and their structures elucidated. The nebularic acids are α,β -unsaturated ketones, the two lactones have an α,β -unsaturated carbonyl moiety, and nebularic acid B in addition has an epoxy group conjugated with a carbonyl group, which means that all four sesquiterpenoids are potentially biologically active compounds (EFSA, 2007). Nebularic lactone A has been shown to exhibit moderate antibacterial activity and nebularic acid B to exhibit both significant antifungal and antibacterial activity (Wangun *et al.*, 2006). The chemical structure of one of these α,β -unsaturated carbonyl compounds, nebularic acid B, is shown below.

Nebularic acid B

Other α,β -unsaturated constituents: The α,β -unsaturated aldehyde, 2,4-diphenyl-2-butenal was isolated in small quantities from fruiting bodies of Clouded Funnel. The compound was shown to have a weak antifungal and antibacterial activity *in vitro*. Injuring fruit bodies by grounding 15 minutes before analysis, resulted in other potentially biologically active α,β -unsaturated carbonyl compounds being formed (Pang *et al.*, 1994). It is not known, whether the α,β -unsaturated constituents (including the sesquiterpenoids) from Clouded Funnel may have adverse effects in humans.

The structural formula of 2,4-diphenyl-2-butenal is shown below.

2,4-Diphenyl-2-butenal

Muscarine: According to some toxicology textbooks on mushrooms, Clouded Funnel may contain muscarine (e.g., Benjamin, 1995; Lincoff & Mitchel, 1977). There are, however, no analytical studies available, confirming that muscarine may occur in this mushroom.

Hydrogen cyanide: Stijve & de Meijer (1999) found traces (less than 1 mg/kg, fresh weight) of hydrogen cyanide in Clouded Funnel. So low levels are of no health concern in relation to consumption of this mushroom.

Recommendation

Clouded Funnel gives rise to intoxication with gastrointestinal distress in some people, even after thorough cooking. The toxicant is not known. It should therefore not be used in commercial trade.

Listing 4

Database search information

SciFinder by December 2012. Keywords: *Lepista nebularis* or *Clitocybe nebularis*. PubMed by December 2012. Keywords: *Clitocybe* or *Lepista* or *Tricholoma*.

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Clitopilus prunulus (Scop.) P. Kumm.

The Miller (DK: Gråhvid Melhat, FI: Jauhosiemi, IS: Voðhöttur, Vatthöttur, NO: Melsopp, SE: Mjölkskivling).

Background and risk assessment

The Miller (*Clitopilus prunulus*) is an edible mushroom.

The Miller is a saprotrophic mushroom. It grows mainly on soil in deciduous and coniferous forests. It is common in temperate and up to the middle boreal areas, but occasional or rare in northern boreal and subarctic/subalpine areas of the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of The Miller, and no natural toxicants constituting a risk for humans have been identified in the mushroom.

Recommendation

The Miller resembles some small Funnel species (*Clitocybe* species) like Fools Funnel (*Clitocybe rivulosa*), which are very poisonous (see Chapter 4). Therefore, the Miller is not regarded as suitable for commercial marketing.

Listing 3

Database search information

SciFinder by February 2012. Keywords: *Clitopilus*.

References

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Coprinopsis atramentaria (Bull.) Redhead, Vilgalys & Moncalvo (*Coprinus atramentarius* (Bull.) Fr.)

Common Inkcap (DK: Almindelig Blækhat, FI: Harmaamustesieni, IS: Slöttblekil, NO: Grå blekksopp, SE: Grå bläcksvamp).

Background and hazard identification

Common Inkcap (*Coprinopsis atramentaria*) is not an edible mushroom.

It is a saprotrophic mushroom having a solitary or fasciculate growth around dead wood. It is very common in temperate to boreal zones, and occasional in subalpine/subarctic zones of the Nordic countries (Knudsen & Vesterholt, 2012).

Several intoxications have been reported after consumption of Common Inkcap, when it is combined with intake of alcoholic beverages. Repeated oral doses of one of its constituents, coprine have given rise to severe damages of germ cells and other adverse effects in male rats similar to those of alkylating compounds. Furthermore, coprine is mutagenic in *in vitro* tests. Therefore, Common Inkcap should not be used for consumption.

Already in the beginning of the 20th century it was observed that consumption of Common Inkcap together with alcoholic beverages could give rise to poisonings with “antabuse” (disulfiram)-like effects with flushing of face, headache, rapid heart rate, nausea and metallic taste

(Alder, 1960; Matthies & Laatsch, 1992; Chilton, 1994; Trestrail, 1991). Nevertheless, it was considered as an edible mushroom, and (unfortunately) in several mushroom textbooks and field guides, it still is, provided that alcohol is not consumed at the same time (e.g., Gerhardt, 1997; Knudsen & Petersen, 2007; Laux, 2001; Lincoff, 2010; Lohmeyer & Künkle, 2006; Roth, 1990).

However, in 1975 Lindberg and colleagues, and independently of them Hatfield and Schaumberg, isolated and structure-elucidated the causative agent, which the two groups agreed to name coprine (Lindberg *et al.*, 1975; Hatfield & Schaumberg, 1975). Subsequently, the structure of coprine was confirmed by synthesis (Lindberg *et al.*, 1977). Coprine is a non-protein amino acid with an unusual structure, including a cyclopropane ring. It can be described as a condensate of cyclopropanone and glutamine. It is a relatively stable compound, which is not destroyed during cooking, but can be hydrolysed to 1-aminocyclopropanol and/or cyclopropanone hydrate in acidic environment or *in vivo* (Lindberg *et al.*, 1977; Wiseman & Abeles, 1979). The structural formulae for coprine, 1-aminocyclopropanol and cyclopropanone hydrate are shown below.

Coprine

1-Aminocyclopropanol

Cyclopropanone hydrate

Hazard characterisation

Human observations: After consumption of Common Inkcap together with alcohol the most common symptoms are very similar to those observed after application of the drug disulfiram (“antabuse”) to discourage alcoholics from drinking alcoholic beverages. Typical symptoms of the Coprinus (“antabuse”) syndrome include flushing of the face, tingling in arms and legs, throbbing headache, tachycardia, sweating, nausea, vomiting, metallic taste, possibly cardiovascular arrhythmia, and hypotension, whereas colic and diarrhoea are rare. The severity of the symptoms depends on the amounts of mushroom and alcohol consumed and the time between these intakes. The first symptoms may appear within 15(5) minutes to 2 hours, generally 3–6 hours after the combined exposure, but the sensitivity to alcohol may persist 2–3 days after consumption of the mushroom. Usually the symptoms last from 30 minutes to a few hours with complete recovery. There are no documented data available on the content of coprine in Common Inkcap or the amount of Common Inkcap and alcohol consumed in the published cases on intoxications (Barceloux, 2008; Benjamin, 1995; Bresinsky & Besl, 1985; Chilton, 1994; Michelot, 1992; Rumack, 1994).

Causative agent: In order to isolate, identify and study the constituents in Common Inkcap responsible for the disulfiram-like effects reported in humans after consumption of the mushroom in combination with alcoholic beverages, several *in vivo* studies have been performed. It was shown that experimental animals such as rats and mice got an elevated level of acetaldehyde in the blood after combined intake of ethanol and Common Inkcap, with some of the same symptoms reported above in humans, e.g. rats had swollen faces and lachrymation and reduced blood pressure (e.g. Coldwell *et al.*, 1969; Hatfield & Schaumberg, 1975; Lindberg *et al.*, 1975; 1977). Studies with coprine administered orally at different doses in combination with ethanol (intraperitoneally injected) and with different time intervals demonstrated the blocking of acetaldehyde dehydrogenase, resulting in different levels of acetaldehyde in the blood. The lowest pharmacologically, active oral dose in rats was found to be approximately 10 mg coprine per kg body weight (Carlsson *et al.*, 1978; Lindberg *et al.*, 1977).

In a 14-day repeated dose oral toxicity study on male rats doses of 20 or 200 mg coprine per kg body weight were applied (Jönsson *et al.*, 1979). At 200 mg per kg, coprine caused severe changes in the testis, probably due to direct effects on the germ cells. Pronounced lymphopenia and thrombocytopenia were also documented at the high dose of coprine and the histological examination showed bone marrow depres-

sion and hypercellularity of spleen, thymus and lymph nodes. At the low dose (20 mg per kg) no treatment-related effects were observed except a small but significant reduction in thrombocyte count. It was noted that the effects caused by coprine are similar to those observed after exposure to alkylating agents. Furthermore, coprine was found to be mutagenic *in vitro* in the Ames test using *Salmonella thyphimurium* as indicator organism for mutagenic activity (industrial report referred to by Jönsson *et al.*, 1979). These observations indicate that it is possible that coprine might be a genotoxic carcinogen, for which it is not possible to give a NOAEL, based on the data available.

Mechanism of acute intoxication: It is rendered probable that coprine inactivates aldehyde dehydrogenase (Hatfield & Schaumberg, 1975; Tottmar & Lindberg, 1977). This, inhibition could cause the effects seen after intake of Common Inkcap and alcoholic beverages (ethanol). However, coprine does not inhibit aldehyde dehydrogenase *in vitro*, and it has been concluded that the active principle must be as metabolite of coprine (Hatfield & Schaumberg, 1975; Tottmar & Lindberg, 1977; Wiseman & Abeles, 1979). Initially coprine may be hydrolysed to 1-aminocyclopropanol and/or cyclopropanone hydrate. Both these compounds have shown activity *in vitro* and *in vivo*. Further metabolism may yield highly reactive metabolites which can react with a thiol group of the dehydrogenase and block the enzyme, increasing the acetaldehyde concentration in blood. This can explain many of the acute intoxication symptoms seen after consumption of Common Inkcap together with alcoholic beverages (Michelot, 1992; Wiseman & Abeles, 1979).

Other bioactive constituents: Two illudins designated C₂ and C₃ have been isolated from cultures of Common Inkcap. Like other illudins, they are sesquiterpenes with an unusual structure, including a spirocyclopropane group. Both compounds have been shown to have antimicrobial effects *in vitro* (Lee *et al.*, 1996). The structures of these two illudins are very similar to the structure of illudin S. This compound is anticipated to give the intoxications observed when Jack O'Lantern (*Omphalotus olearius*) is consumed (particularly in middle and southern Europe) (Bresinsky & Besl, 1985). It is not known to what extent illudin C₂ and C₃ are formed in the fruit bodies of the Common Inkcap and whether they, like illudin S in Jack O'Lantern, are toxic to humans. The structural formula of one of these sesquiterpenoids, illudin C₂ is shown below.

Illudin C₂

Exposure

Common Inkcap is reported to contain on average 160 mg coprine per kg (fresh weight), more in older mushrooms and half as much in young ones (Lindberg *et al.*, 1977). Matthies and Laatsch (1992) reported 160–360 mg coprine per kg fruit body (fresh weight). Coprine has also been found in other, but not edible *Coprinopsis* species and may also occur in relatively small amounts, approximately 10–15 mg per kg (fresh weight) in the edible Shaggy Inkcap (*Coprinus comatus*) (Matthies & Laatsch, 1992). However, there are no data available on the content of coprine in Common Inkcap or the amount of Common Inkcap and alcohol consumed in the published cases on intoxications.

Risk characterization

In animal studies, the lowest pharmacologically active oral dose of coprine, 10 mg/kg body weight in rats and a NOAEL below 20 mg/kg body weight has been identified in two-week oral studies in rat. It is, however, indicated that coprine acts similarly to alkylating agents and that it is mutagenic in bacterial tests. Therefore, it cannot be excluded that coprine is a genotoxic carcinogen, and based on the studies available, it is not possible to give a safe level of intake of Common Inkcap.

Recommendation

Common Inkcap may give rise to acute intoxications when consumed together with alcoholic beverages. Furthermore, the causative constituent, coprine, has in experimental studies been shown to damage germ cells and to have other adverse effects, possibly including the potential to induce irreversible long term effects such as cancer. Common Inkcap should not be considered as an edible mushroom.

Listing 4

Database search information

SciFinder by January 2012. Keywords: *Coprinus* or *Coprinopsis*. SciFinder by August 2012. Keywords: Coprine. PubMed by December 2012. Keywords: *Coprinus* or *Coprinopsis* and *atramentarius* or *atramentaria*.

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Coprinus atramentarius (Bull.) Fr.

See *Coprinopsis atramentaria*.

Coprinus comatus (O.F.Müll.) Pers.

Shaggy Inkcap, Lawyer's Wig, (Shaggy Mane) (DK: Stor Parykhat (Paryk-Blækhat), FI: Suomumustesieni, IS: Ullblekill, NO: Matblekksopp, SE: Fjällig bläcksvamp).

Background and risk assessment

Shaggy Inkcap (*Coprinus comatus*) is an edible mushroom.

It is a saprotrophic mushroom growing in open land, lawns, stubble-fields and other fertilized areas, also in forests. It is very common in temperate to boreal zones, and occasional in subarctic to alpine zones of the Nordic countries (Knudsen & Vesterholt, 2012).

Some reports describe rather unspecific poisonings after consumption of Shaggy Inkcap – and these descriptions are not always well-documented. It is not always clear, if the intoxications have occurred in combination with alcohol consumption. Symptoms such as gastrointestinal distress and chills have begun within 0.5–8 hours after consumption of the mushroom (Beug, 2009; 2012; Beug *et al.*, 2006; Trestrail, 1991). Some bioactive constituents have been identified in Shaggy Inkcap:

Bioactive constituents

Coprine: The causative agent has not been identified, but at least in some of the cases, it has been suggested to be the small amounts of coprine which may occur in Shaggy Inkcap. Michelot (1992) in his thorough review on coprine poisonings refers to a level of 26 mg coprine per kg mushroom (fresh weight) based on data from Laatsch (1990). However, Matthies & Laatsch (1992) reports a level of approximately 10–15 mg coprine/kg Shaggy Inkcap (fresh weight). The presence of coprine was confirmed by comparing the compound isolated from the mushroom with authentic coprine using mass spectrometry.

Coprine has been shown to damage germ cells and to give other toxic effects in experimental animals and in combination with alcohol to give disulfiram(antabuse)-like effects in humans. Whether the relatively small amounts of coprine which may occur in Shaggy Inkcap have the potential to raise concern in relation to antabuse-effects or the toxic effects in animals is not known (for further details on coprine toxicity, see *Coprinopsis atramentaria*).

Lectins: Mikiashvili *et al.* (2006) have demonstrated lectin activity in extracts from fruit bodies of Shaggy Inkcap on rabbit erythrocytes. However, the lectin is not well characterized with respect to binding capacity, stability or to molecular mass.

Statins: Small amounts of lovastatin have been identified in cultured mycelia of Shaggy Inkcap. Chen *et al.* (2012) reported an average level of 110 mg/kg dry weight (mean of 3 samples), whereas Lin *et al.* (2013) reported a level of 1.2 mg /kg dry weight (mean of 3 samples). For further data on statins, see *Pleurotus ostreatus*.

Ergothioneine: Relatively small amounts of ergothioneine were demonstrated by List (1957) who isolated approximately 60 mg per kg of fruit bodies of Shaggy Inkcap (dry weight), and by Chen *et al.* (2012) who found 399 mg (mean of 3 samples) and by Lin *et al.* (2013) who found 67 mg (mean of 3 samples) per kg of mycelia (dry weight). For further data on ergothioneine, see *Lepista nuda*.

Furan derivatives: Luo *et al.* (2007) have isolated six nematicidal furan and hydrogenated furan derivatives from cultivated mycelia. There are no studies on occurrence in the fruit bodies, and the potential biological effects in humans are unknown.

Mutagens: Sterner *et al.* (1982) investigated the mutagenic activity of an extract of Shaggy Inkcap in the Ames test, using the *Salmonella typhimurium* strains TA98, TA100 and TA2637 as indicators. The extract was positive in all three *Salmonella* strains but the activity was not influenced by introducing a metabolizing rat microsomal system in the test

assay. Unfortunately, Sterner *et al.* (1982) give no information on whether they had controlled for the potential presence of histidine in the extract, as it is well known that false positives may be obtained in the presence of this amino acid. Also Morales *et al.* (1990) studied Shaggy Inkcap extracts in the Ames test. Extracts of freshly harvested mushrooms were reported to be weakly mutagenic in the *Salmonella typhimurium* strains TA98 and TA100 (less than doubling in mutation frequency). Apparently, Morales *et al.* (1990) did not control for possible histidine in the extracts for the Ames test. It is concluded that there is no strong indication that extracts of Shaggy Inkcap are mutagenic.

Recommendation

No special recommendation.

Listing 2

Database search information

SciFinder by September 2012. Keywords: *Coprinus*, coprine. PubMed by December 2012. Keywords: *Coprinus comatus*.

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Cortinarius caperatus (Pers.) Fr. (*Rozites caperatus* (Pers.) P. Karst.)

The Gypsy (DK: Klidhat, FI: Kehnäsieni, IS: Hrukkuhössveppus, NO: Rimsopp, SE: Rimskivling (Rynkad tofsskivling)).

Background and risk assessment

The Gypsy (*Cortinarius caperatus*) is an edible mushroom.

It forms mycorrhiza in coniferous forests with Spruce (*Picea*) and Pine (*Pinus*) and in deciduous forests with Beech (*Fagus*) and Oak (*Quercus*), in subalpine forests with Dwarf Birch (*Betula nana*). It is rare in Denmark and Iceland, but otherwise common in the Nordic countries (Knudsen & Vesterholt, 2012).

The Gypsy is distinguished from all other Webcap (*Cortinarius*) species, as it has a distinct ring. The ring is protruding and comes off easily (Knudsen & Vesterholt, 2012).

No intoxications have been reported after consumption of The Gypsy and no natural toxicants constituting a risk for humans have been identified in the mushroom.

Grüter *et al.* (1991) have demonstrated a weakly mutagenic activity of extracts of The Gypsy using a histidine-independent bacterial system. Whether the mutagens are destroyed during food preparation is unknown.

Recommendation

No special recommendation.

Listing 2**Database search information**

SciFinder by February 2012. Keywords: *Rozites caperatus*, *Cortinarius caperatus*.

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Cortinarius, other species, e.g., *C. armillatus* (Fr.) Fr

Webcap species e.g. Red Banded Webcap (DK: Visse slørhatte, fx Cinnoberbæltet Slørhat, FI: *Cortinarius* lajin seitikit, esim. Punavyöseitikki *Cortinarius*, IS: Einstaka kögrar t.d. no Icelandic name, NO: Slørsopper f. eks. Rødbelteslørsopp, SE: *Cortinarius*-arter, t.ex. rödbandad spindling, Vissa spindlingar (Spindelskivlingar)).

Background and risk assessment

Webcap (*Cortinarius*) is the biggest genus of fungi in the Nordic countries with 369 species described in Funga Nordica and, based on phylogenetic studies, it is assumed that at least 900 species occur in the Nordic countries (Knudsen & Vesterholt, 2012). The Webcap species form mycorrhiza with all kinds of deciduous and coniferous trees. With few exceptions, e.g., The Gypsy (*C. caperatus*), most of the Webcap species are difficult to identify for non-specialists. Some Webcab species are deadly poisonous, e.g., Deadly Webcap (*Cortinarius rubellus*) and Fool's Webcap (*C. orellanus*), several are suspicious, e.g., species from the section *Dermocybe*, containing anthraquinones, some of which are laxative and/or mutagenic *in vitro* (Barceloux, 2008; von Wright *et al.*, 1992; Besl *et al.*, 1978), and other Webcap species indicated to contain orellanine, the toxicant in Deadly Webcap and in Fools Webcap (Oubrahim *et al.*,

1997; Danel *et al.*, 2001; Matthies & Laatsch, 1991). The edibility of most Webcap species is unknown and only a few are reported as edible, e.g. Red Banded Webcap (*C. armillatus*) (Gerhardt, 1997; Knudsen & Petersen, 2007; Lohmeyer & Künkele, 2006).

Red Banded Webcap forms mycorrhiza in deciduous and mixed forests. It is rare in temperate zone, but very common in hemiboreal and subalpine/subarctic zones of the Nordic countries (Knudsen & Vesterholt, 2012).

No intoxications have been reported after consumption of Red Banded Webcap. However, it contains several anthraquinone derivatives, endocrocin, dermolutein and dermorubin (Besl *et al.*, 1978). The structural formula of one of the major anthraquinones in Red Banded Webcap, dermolutein, is shown below.

Dermolutein

Recommendation

Many webcaps (*Cortinarius* species) are difficult to identify, and some of them are deadly poisonous, e.g., Deadly Webcap (*Cortinarius rubellus*) (See Chapter 4). Webcap species are therefore, with the exception of The Gypsy (*C. caperatus*), not regarded as suitable for commercial marketing.

Database search information

SciFinder by February 2012. Keywords: *Cortinarius armillatus*.

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Craterellus cornucopioides (L.) Pers. (*Cantharellus cornucopioides* (L.) Fr.)

Horn of Plenty (DK: Trompetsvamp, FI: Mustatorvisieni, IS: Svartlúður, NO: Svart trompetsopp, SE: Svart trumpetsvamp).

Background and risk assessment

Horn of Plenty (*Craterellus cornucopioides*) is an appreciated, edible mushroom.

It is a common in rich deciduous forests, with Beech (*Fagus*) and Oak (*Quercus*) in Denmark and in southern parts of Finland, but occasional or rare in northern parts of Finland, Norway and Sweden with Hazel (*Corylus*) and Spruce (*Picea*). It is occasional in Iceland and can be found, most abundantly in the late autumn (Hansen & Knudsen, 1997). It is available on the market both as fresh and dried.

There are no intoxications reported after consumption of Horn of Plenty. However, some potentially bioactive compounds may occur in Horn of Plenty.

Bioactive constituents

Ketoesters: Three ketoesters have recently been identified in Horn of Plenty: 4-oxohex-1,6-diyl diacetate, 4-oxohex-5-enyl acetate and 4-oxo-6-hexyl acetate (Liu *et al.*, 2010). The structures of these esters do not indicate concern with respect to potential toxicity.

Sesquiterpenes: It should be remarked that four unusual sesquiterpenes (craterellins) have been isolated from cultures of the related *Craterellus odoratus*. These constituents have inhibitory effects against 11 β -hydroxysteroid dehydrogenases (Zhang *et al.*, 2010).

Recommendation

It should be examined whether Horn of Plenty (*C. cornucopiodes*), like *C. odoratus*, contains craterellins and if so, in which amounts?

Database search information

SciFinder by December 2012. Keywords: *Craterellus*, *Cantharellus*.

Listing 1

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Craterellus lutescens (Pers.) Duby (*Craterellus lutescens* (Fr.) Fr., *Cantharellus lutescens* Fr.)

Yellow Foot (DK: Rødgul Kanterel/Rødgul Trompetsvamp, FI: Kosteikko-vahvero, IS: Gulllúður, NO: Gul trompetkanarell/Gul trompetsopp, S: Rødgul trumpetsvamp).

Background and risk assessment

Yellow Foot (*Craterellus lutescens*) is an appreciated edible mushroom.

It is not found in Denmark. It grows with Spruce (*Picea*) on calcareous soil and is occasional or rare in hemiboreal and boreal zones of Finland, Norway and Sweden and common in southern parts of Sweden (Hansen & Knudsen, 1997). It is available on the market, mainly as fresh.

Consumption of Yellow Foot is not reported to give rise to intoxications.

Due to its genetic relationship to the Trumpet Chanterelle (*C. tubaeformis*) and, therefore, anticipated compositional similarity, it is possible that *C. lutescens* by injury may form (8E)-10-hydroxy-8-decenoic acid. This compound has been shown to be formed in injured Trumpet Chanterelle (Anke *et al.*, 1996; Pang *et al.*, 1992). By damage of the mushroom it is anticipated to be formed enzymatically from linoleic acid, which is an important fatty acid in the fresh mushroom. Further information on the biological activity of (8E)-10-hydrox-8- decenoic acid is given in the monograph on

Trumpet Chanterelle. (8E)-10-Hydroxy-8-decenoic acid is not significantly degraded in boiling water (Anke *et al.*, 1996).

Recommendations

Yellow Foot (*Craterellus lutescens*) may like the closely related Trumpet Chanterelle (*Craterellus tubaeformis*) form weakly mutagenic compounds if injured.

Listing 1

Database search information

SciFinder by December 2012. Keywords: *Craterellus*, *Cantharellus*.

References

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Craterellus tubaeformis (Fr.) Quél.
(*Cantharellus tubaeformis* (Bull.) Fr., *C. tubaeformis* Fr.)

Trumpet Chantarelle (“Chanterelle gris”, “Winter Chanterelle”)
(DK: Tragt-Kantarel, FI: Suppilovahvero, IS: Grálúður; NO: Traktkantarell, SE: Trattkantarell).

Background and risk assessment

Trumpet Chanterelle (*Craterellus tubaeformis*) is an appreciated edible mushroom.

It is commonly found in the Nordic countries, except in Iceland, in both coniferous and deciduous forests with Spruce (*Picea*) and Beech (*Fagus*) (Hansen & Vesterholt, 1997). It is marketed both as fresh and dried.

Consumption of Trumpet Chanterelle is not reported to have given rise to intoxications.

When injured, the Trumpet Chanterelle may form the fatty acid derivative (8E)-10-hydroxy-8-decenoic acid in amounts of up to 50 mg per kg fresh weight (Anke *et al.*, 1996; Pang *et al.*, 1992).

(8E)-10-Hydroxydec-8-enoic acid

This fatty acid derivative is anticipated to have the potential to be biologically oxidized to the corresponding α , β -unsaturated aldehyde, having a structural alert for genotoxic activity (EFSA 2007). In fact, (8E)-10-hydroxy-8-decenoic acid has been shown to be a very weak directly acting mutagen when tested in *Salmonella typhimurium* strain TA100 in the absence of S-9 mix, whereas the compound was negative in this test strain with metabolic activation (S-9 mix) and in strain TA98 with and without S-9 mix (Anke *et al.*, 1996). (8E)-10-Hydroxy-8-decenoic acid shows very weakly antimicrobial and cytotoxic activities in a few of the altogether seventeen bacteria, fungi and cell lines tested (Anke *et al.*, 1996). The acid has also shown weakly nematocidal activity (Stadler *et al.*, 1994). (8E)-10-Hydroxy-8-decenoic acid is not significantly degraded in boiling water (Anke *et al.*, 1996).

Recommendations

Trumpet Chanterelle (*C. tubaeformis*) may form weakly mutagenic compounds if injured.

Listing 1

Database search information

SciFinder by December 2012. Keywords: *Craterellus*, *Cantharellus*.

References

- Anke H, Morales P & Sterner O (1996): Assays of the biological activities of two fatty acid derivatives formed in the edible mushrooms *Cantharellus cibarius* and *C. tubaeformis* as a response to injury. *Planta Medica* 62: 181–183.
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Flammulina velutipes (Curtis) Singer

Velvet Shank, (Enoki-take, Golden Needle Mushroom) (DK: Almindelig Fløjlsfod ("Enoki"), FI: Talvijuuurekas, IS: Veturfönungur (Loðfótur), NO: Vintersopp ("Enoki" og "Enokitake"), SE: Vinterskivling (Vinternagelskivling)).

Background and risk assessment

Velvet Shank (*Flammulina velutipes*) is an edible mushroom.

It grows on dead or living wood or roots of deciduous trees, rarely on conifers, mainly in late autumn or winter. It is very common in temperate and hemiboreal, common in boreal/subalpine/subarctic and occasional in arctic/alpine zones of the Nordic countries (Knudsen & Vestersholt, 2012).

Commercially, only the cultivated forms of Velvet Shank are of importance. It is mainly cultivated in south-eastern parts of Asia, but also to some extent in e.g. the Nordic countries.

There are no intoxications reported after consumption of Velvet Shank except for a questionable case (Beug *et al.*, 2006). However, it contains different potentially bioactive compounds:

Bioactive constituents

Ergothioneine: Velvet Shank contains 57 mg (Lin *et al.*, 2013) or 455 mg of ergothioneine per kg of dried mushroom (Chen *et al.*, 2012) (for further data on ergothioneine, see *Lepista nuda*).

Statins: Velvet Shank contains lovastatin. Lee *et al.* (2006) reported, 4–5 mg – and Chen *et al.* (2012) reported 91 mg per kg dried fruit bodies (Chen *et al.*, 2012). (for further data on lovastatin, see *Pleurotus ostratus*).

Ribosome inactivating proteins are enzymes that depurinate tRNA's. This depurination inactivates the ribosomes, thus inhibiting protein synthesis (Stirpe & Battelli, 2006; Nielsen & Boston, 2001). At least four ribosome inactivating proteins have been characterized in Velvet Shank: Flammulin (molecular mass 40 kDa; velutin (molecular mass 13.8 kDa), flamin (molecular mass 30 kDa) and velin (molecular mass 19 kDa). These four proteins all inhibit translation in a rabbit reticulocyte lysate system (Ng & Wang, 2004). Generally, ribosome inactivating proteins have been shown to have various biological activities e.g. antimitogenic, antiproliferative, and antifungal effects in *in vivo* and *in vitro* experimental test systems (Lam & Ng, 2001). There are no data available demonstrating whether these proteins in Velvet Shank have any biological effects in relation to consumption of the mushroom.

Cytolytic proteins: A cytolytic protein, flammutoxin has been isolated from Velvet Shank. It causes significant electrocardiographical changes when administered parenterally to cats. It also gives rise to swelling and inhibition of respiration of Ehrlich ascites tumour cells (Lin *et al.*, 1974; 1975) and hemolyses human as well as many different experimental animal erythrocytes (Lin *et al.*, 1975; Bernheimer & Oppenheim, 1987). Tomita *et al.* (1998) and Tadjibaeva *et al.* (2000) did show that flammutoxin has a molecular mass of 31 kDa, and that it assembles into a ring-shaped oligomer on target cells, e.g. membranes of erythrocytes. As heat-treatment at temperatures higher than 60 °C for 30 minutes completely abolished the hemolytic activity of flammutoxin (Lin *et al.*, 1975) it is also likely, it is destroyed during cooking. It may also be inactivated in or not absorbed from the gastrointestinal tract.

Lectins: A dimeric lectin with a molecular mass of 20 kDa has been isolated from Velvet Shank and characterized. It consists of two subunits of 8 and 12 kDa. Only the 12 kDa subunit exhibits hemagglutinating and mitogenic activity. The hemagglutinating effects are reduced at temperatures beyond 60 °C and are undetectable above 80 °C. As the lectin in Velvet Shank apparently will be destroyed during cooking, the content of lectin in the mushroom is of no health concern.

Recommendation

No special recommendation.

Listing 1 (as cultivated), Listing 2 (as wild)

Database search information

SciFinder by January 2012. Keywords: *Flammulina*.

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Gomphidius glutinosus (Schaeff.) Fr.

Slimy Spike (DK: Grå Slimslør, FI: Limanuljaska, IS: Slímgumpur (Slímstautull), NO: Sleipsopp, SE: Citronslemskivling (Citrongul slemskivling)).

Background and risk assessment

Slimy Spike is an edible mushroom.

It is mycorrhizal with Spruce (*Picea*). It is very common in the Nordic countries up to the subalpine/subarctic zones, where it is rare, but locally occasional (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Slimy Spike, and no natural toxicants constituting a risk for humans have been identified in the mushroom.

Recommendation

No special recommendation.

Listing 2

Database search information

SciFinder by February 2012. Keywords: *Gomphidius*.

References

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Gomphus clavatus (Pers.) Gray

Pig's Ear (DK: Køllekantarel, FI: Pölkkyieni, IS: Fjólusteðji, NO: Fiolgubbe, SE: Violgubbe).

Background and risk assessment

Pig's Ear (*Gomphus clavatus*) is an edible mushroom.

It grows preferably on calcareous soil in coniferous, rarely deciduous forests. It is not found in Iceland, is occasional in parts of Norway and rare in Denmark, Finland and Sweden (Hansen & Knudsen, 1997).

There are no intoxications reported after consumption of Pig's Ear, and no natural toxicants constituting a risk for humans have been identified in the mushroom. In a study on cantharelloid mushrooms the toxicant norcaperatic acid was found in high amounts, 2–4 g/kg fresh weight in two related *Gomphus*-species, *G. floccosus* and *G. kauffmanii*, but not in Pig's Ear (Henry & Suillivan, 1969).

Recommendation

Red-listed in Denmark and in Sweden as vulnerable and in Norway as near threatened.

Should only be marketed in the Nordic countries, if it is from countries, where it is not red-listed.

Listing 2

Database search information

SciFinder by February 2012. Keywords: *Gomphus clavatus*.

References

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Grifola frondosa (Dicks.) Gray

Hen of the Woods (DK: Tueporesvamp, FI: Koppelokääpä, IS: Blöðkubora, NO: Korallkjuke, SE: Korallticka).

Background and risk assessment

Hen of the Woods (*Grifola frondosa*) is an edible mushroom.

It is a weakly parasitic mushroom growing at the basis of living trees, especially Oak (*Quercus*), but occasionally also as a saprotroph on decaying wood. It is not found in Iceland and is otherwise rare in the Nordic countries (Hansen & Knudsen, 1992). It is cultivated, especially in south-eastern parts of Asia, where it is a very popular culinary and medicinal mushroom.

There are no intoxications reported after consumption of Hen of the Woods, except for one very special and not well documented case (Gonmori & Yokoyama, 2009). This was a female in her late 40's, who had received dialysis for more than 35 years and was hospitalized due to illness. During the hospital stay she ate two cooked meals at different days with 5 g and 10 g of Hen of the Woods, respectively. Some 14–18 days after these meals, she developed cramps and fell into coma. Ten days later she died. The authors suggest, that hydrogen cyanide from the mushroom could have been the causative agent. This is found unlikely, as described below.

The role of Hen of the Woods in this intoxication, and which constituent could have contributed to the fatality, is not clear. Some bioactive constituents have been isolated from the mushroom:

Bioactive constituents

Cyanogenic compounds: Hen of the Woods contains small amounts of cyanogenic compounds, on average corresponding to 13 mg hydrogen cyanide per kg fresh mushroom (n = 5, range 1.8–46 mg per kg) (Shindo *et al.*, 1999). Such small amounts of hydrogen cyanide are not of concern in relation to consumption of the mushroom. Furthermore, a significant fraction of the hydrogen cyanide will disappear during cooking (Stijve & de Meijer, 1999).

Lectins: An N-acetylgalactosamine-specific lectin was isolated from the fruit bodies of Hen of the Woods. Gel filtration using various buffers and matrices showed it had a molecular mass between 30 and 52 kDa, whereas determination by gel electrophoresis indicated 33, 66 and 100 kDa. A faint band was also observed at 65 kDa. The isolated lectin agglutinated human erythrocytes regardless of blood type. The hemagglutinating activity was highest at pH 4.5. The lectin was cytotoxic against HeLa cells (Kawagishi *et al.*, 1990). As no information on the thermostability of the lectin is available, it is difficult to estimate whether the lectin could be of any concern in the cooked mushroom.

Statins: Traces of lovastatin (3.2 mg/kg dried biomass) were detected in the fruit bodies of Hen of the Woods by Lin *et al.* (2013), whereas Chen *et al.* (2012) were unable to detect the compound (for further data on statins, see *Pleurotus ostreatus*).

Ergothioneine: High but various amounts of ergothioneine have been demonstrated in the mushroom. Dubost *et al.* (2006) found the average level in fruit bodies to be 1,840 mg/kg dry weight (n=9), whereas Chen *et al.* (2012) reported 553 mg/kg dry weight (n=3) and Lin *et al.* (2013) 143 mg/kg dry weight (n=3). Chen *et al.* (2012) also analysed the mycelia and found 296 mg/kg (dry weight, n=3). For further data on ergothioneine see *Lepista nuda*.

Recommendation

Red-listed in Denmark, Norway and Sweden as near threatened. Should only be marketed in the Nordic countries, if it is from countries, where it is cultivated or not red-listed.

Listing 1

Database search information

SciFinder by August 2012. Keywords: *Grifola*. PubMed by August 2012.

Keywords: *Grifola frondosa*.

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Gyromitra esculenta (Pers.) Fr.

False Morel, (Turban, Brain Mushroom) (DK: Spiselig Stenmorkel, FI: Korvasieni, IS: Krymplusveppur, Krymplumyrkill, NO: Sandmorkel, SE Stenmurkla).

Background and hazard identification

False Morel (*Gyromitra esculenta*) is a toxic mushroom.

It is a saprotroph and grows in sandy coniferous woods in the spring-time. It is not found in Iceland, is uncommon in Denmark, but more common in the other Nordic countries. In Finland more than 100 tons are marketed some years. It also grows in Central and Eastern Europe, Asia and North America.

False Morel is commercially available in several countries as fresh, canned or dried mushroom, generally together with a warning that it is needed to pre-treat the mushroom to reduce the content of toxicants before consumption.

The toxicity of False Morel has been known for centuries. Acute intoxications have occurred after consumption of raw, dried or insufficiently cooked mushrooms, but there are also reports on intoxications after consumption of thoroughly pre-treated mushrooms (boiled in large volumes of water for five minutes twice, water being discarded each

time or dried for at least one month). More than 100 fatal cases have been described (Beug, 2006; 2008; Beug *et al.*, 2006; Bianchi *et al.* 1999; Franke *et al.*, 1967; Leathem & Dorran, 2007; Liang *et al.*, 1998; Vitte *et al.*, 1997). However, in several countries, it is considered edible and delicious after thorough pre-treatment.

The toxic constituents in False Morel were elucidated in the 1960s and 1970s, and were shown to be gyromitrin (ethylidene gyromitrin) and eight other hydrazones, and their degradation products/metabolites N-methyl-N-formylhydrazine (MFH) and monomethylhydrazine (MMH).

The structural formulae of the most abundant hydrazone in the False Morel, gyromitrin, and the two hydrazines MFH and MMH formed on degradation/metabolism are shown below.

The content of gyromitrin in False Morel depends on the environmental conditions at the site of growth and the conditions during storage (Andary & Privat, 1985; Andersson *et al.*, 1985; Stijve, 1978). The content of gyromitrin and its degradation product/metabolite MMH has been studied in fresh, dried, canned and parboiled False Morel. Stijve (1978) demonstrated that the greater part of gyromitrin and probably also MFH is chemically bound in the mushroom. Therefore earlier studies using for example diethyl ether to extract the hydrazones/hydrazines only analysed the volatile, not chemically bound minor fraction of the hydrazones/hydrazines in False Morel. This has subsequently been confirmed by several authors, and, consequently, methods for their analysis have been modified (Andary *et al.*, 1984a; Chilton, 1994; Larsson & Eriksson, 1989).

Fresh False Morel has been reported to contain between 40 and 760 mg gyromitrin per kg fresh mushroom. Fresh False Morel collected in France contained 109–760 mg gyromitrin (measured as 50–350 mg total MMH in 7 samples) per kg fresh mushroom (Andary, 1984b), while mushrooms collected in Sweden contained 87–326 mg gyromitrin (measured as 40–150 mg total MMH in 14 samples) per kg of fresh mushroom (Larsson & Eriksson, 1989).

Pyysalo & Niskanen (1977) demonstrated that gyromitrin (ethylidene gyromitrin) constitutes 85–90% of the total amount of diethyl ether extractable hydrazones in False Morel. In total 9 different hydrazones were found; the remaining 8 hydrazones (10–15%) were identified as propylidene, butylidene, 3-methylbutylidene, pentylidene, hexylidene, octylidene, E-2-octenylidene and Z-2-octenylidene gyromitrin. No investigations have studied whether also the 8 minor hydrazones, like gyromitrin, may occur as chemically bound constituents in False Morel, but this is likely.

Air-dried False Morel has been reported to contain very different amounts of gyromitrin, possibly depending on the original content in the fresh mushroom and on the drying conditions. Usually, about 30–80% may be removed by drying (Andary *et al.*, 1985; Larsson & Eriksson, 1989). Typically, levels corresponding to 650–1,760 mg gyromitrin (measured as 300–810 mg total MMH) have been found (Andary *et al.*, 1985; Larsson & Eriksson, 1989), although commercially dried samples have contained up to 2,170 mg gyromitrin (1,000 mg total MMH) (Andary, 1984b) or up to 2,600 mg gyromitrin (1,200 mg total MMH) (Larsson & Eriksson, 1989). Contrary to most studies on gyromitrin/MMH in dried False Morel, Arshadi *et al.* (2006) reported very low levels in an apparently pooled sample of about 220 g of air-dried False Morel obtained locally in Sweden, of 22 mg MMH, corresponding to 48 mg gyromitrin per kg dry weight. However, this study reports very low recovery of MMH in the sample(s), the quantitative aspects are not well described and the validation of the method used is incomplete.

Canned False Morel was studied by Larsson and Eriksson (1984). Fifteen samples showed levels in the range 6–66 mg gyromitrin (measured as 3–30 mg MMH).

Parboiled False Morel (5 samples boiled twice for 5 minutes and water discarded each time) that originally contained 44–130 mg MMH per kg dry weight, corresponding to 95–282 mg gyromitrin per kg dry weight, after boiling contained 13–27 mg MMH, corresponding to 28–59 mg gyromitrin per kg dry weight. Thus, the properly pre-treated False Morel still contains in average 10% of the gyromitrin/MMH content in the pretreated mushroom (Larsson & Eriksson, 1989).

The occurrence of hydrazine derivatives in False Morel is of concern because the majority of known hydrazines, beyond being acutely toxic, have been found to be carcinogenic in experimental animals. Subsequent experiments substantiated the concern as *peroral* studies in rodents with False Morel hydrazones, with MMH or MFH, or the mushroom itself did result in significantly increased tumour frequencies in the dosed

animals compared to the control animals as reviewed by Andersson *et al.* (1995). For a comprehensive review on “Hydrazines and cancer”, see Toth (2000).

The International Agency for Research on Cancer (IARC) concluded in 1983 “Results on studies on gyromitrin itself, supported by studies on two of its metabolites provide sufficient evidence for the carcinogenicity of gyromitrin in experimental animals. No data on humans were available.” (IARC, 1983) and in 2012 that gyromitrin is “Not classifiable as to its carcinogenicity to humans” (IARC, 2012).

The Nordic report “Hydrazones in the False Morel” was published in 1995 and contains a risk assessment of consumption of False Morel (Andersson *et al.*, 1995). Data and conclusions from this report are summarized below and form the basis of the present updated risk assessment.

Hazard characterization

The Nordic report gives information on the hydrazones occurring in False Morel, their absorption and metabolism, acute toxicity, sub-chronic toxicity, developmental toxicity, DNA-binding and genotoxicity, and carcinogenicity. In addition, it reviews the influence of the intact mushroom on these parameters (Andersson *et al.*, 1995). Together with more recent information, the data in the Nordic report are summarized in the following.

Absorption and metabolism

In False Morel gyromitrin, and probably also MFH, can be found in both free and bound form as mentioned above. Gyromitrin rapidly hydrolyses to MFH and acetaldehyde. This takes place spontaneously at room temperature and in the stomach, when False Morel is consumed. MFH is further hydrolysed under physiological conditions to form MMH and formic acid, but this takes place at a slower rate. Experimental studies have shown that firmly bound gyromitrin is very resistant to boiling and drying. However, studies with simulated gastric fluid indicate that the bound gyromitrin can at least partly be liberated during digestion in the stomach. This implies that a mixture of unhydrolysed gyromitrin and other hydrazones, MFH and MMH may be available for absorption from the gastrointestinal tract. It is not known how the intestinal bacterial flora influences the metabolism of these compounds. Hydrazine derivatives are reported to be well absorbed from the gastrointestinal tract, but otherwise, there are only limited experimental data available on the fate of these hydrazones and hydrazine derivatives in the body of both animals and humans.

After absorption the two hydrazines MFH and MMH, which are believed to be the active toxic agents derived from the mushroom constituents, may react directly with carbonyl group-containing cell components like folic acid and pyridoxal 5-phosphate, the active form of vitamin B6. The reaction between these molecules produces a hydrazone that is a powerful inhibitor of the phosphokinase required for phosphorylation of pyridoxal, and it therefore depletes the cell of pyridoxal 5-phosphate. The depletion of active vitamin B6 might lead to glutamic acid decarboxylase inhibition and decrease of γ -aminobutyric acid (GABA) synthesis, with profound effect on the central nervous system. Specific treatment of poisoned patients with neurological symptoms is a high dose of pyridoxine hydrochloride. MFH and MMH can also, as other unsymmetric hydrazines, be metabolised to electrophilic diazonium and carbonium ions. These very reactive metabolites may react with cellular macromolecules like DNA and with cytochrome P-450. The anticipated metabolic pathways are described in more details in the Nordic report (Andersson *et al.*, 1995).

Notably, a reaction detoxifying MFH competes with its activation to reactive molecules. Instead of being activated to MMH, the compound can be acetylated and thereby be protected from being activated. As there are various variants of the acetylating P450 enzyme, having different activities, consumers may carry different alleles of the genes coding for detoxifying enzymes and be more or less prone to be damaged after consumption of False Morel. This may at least partly explain the different sensitivity of consumers to the toxic constituents of False Morel observed.

Acute toxicity

The acute intoxication of humans by False Morel may be biphasic. The first phase, the gastrointestinal phase, usually starts 4–8 hours after the consumption but sometimes as early as after 2 hours and occasionally as late as 24 hours after consumption (Diaz, 2005; Karlson-Stiber & Persson, 2003). This phase is characterized by a feeling of being bloated, stomach pain, nausea, continuous vomiting and on occasion watery or bloody diarrhoea. Other common symptoms are tiredness, headache and dizziness. Most cases do not go beyond this phase and recovery begins after 2 to 6 days. In the more severe cases a second phase follows after a symptom-free period, this being a hepatorenal phase with symptoms of liver damage and sometimes also of hemolysis. In addition serious neurological symptoms may appear. Two to three days into the second phase circulatory collapse and respiratory arrest may lead to death while in coma.

A fatal dose of gyromitrin has been estimated to 10–30 mg gyromitrin per kg body weight for children and 20–50 mg gyromitrin per kg body weight for adults (Flammer, 1985).

Subchronic toxicity

Very little is known about the sub-chronic toxicity of the hydrazone and hydrazine constituents of False Morel in experimental animals and man.

Developmental toxicity

Teratogenicity studies have been performed with MMH in female rats. These studies showed a clear dose-dependent decrease in pregnancy rate and an increase in the number of resorptions in the exposed animals as compared to the controls. The "Lowest Observed Effect Level" (LOEL) for MMH in this study was 3.0 mg MMH/kg body weight/day after intravenous infusion and 5.0 mg MMH/kg body weight/day after *peroral* application.

DNA-binding and genotoxicity

Studies have shown that the mushroom hydrazones and degradation products are able to induce DNA lesions, probably via a reactive methyl radical. Among the lesions identified are N-7-methylguanine and O⁶-methylguanine; the latter type of lesion being correlated to mutagenic and carcinogenic activity.

Carcinogenicity

A series of long-term *peroral* carcinogenicity studies in mice have been performed with gyromitrin, three other hydrazones present in False Morel, their degradation products/metabolites MFH and MMH and False Morel itself. In all studies, when fed to the experimental animals, the hydrazine/hydrazone constituents as well as False Morel itself gave rise to significantly increased tumour frequencies as compared to the tumour frequencies in non-treated animals.

It should be underpinned, that the carcinogenic studies have not been performed in accordance with approved standard protocols for carcinogenicity studies. The diets for experimental animals were not always balanced, and the animals were exposed to the hydrazones/hydrazines/mushroom until they died or were in poor condition instead of for a pre-determined length of time. In addition, the control groups were usually not started at the same time as the treated groups. Although at least three dose levels are recommended in carcinogenicity studies in order to identify potential dose-response effects, this requirement was only partially fulfilled.

Though the design of these carcinogenicity studies are not of satisfying standard, the accumulated data indicate that the hydrazine and hydrazone derivatives in False Morel (gyromitrin and three other hydrazones, MFH and MMH), as well as False Morel itself, are carcinogenic in experimental animals. The lowest observed effect level "LOEL" for MMH

(sulfate) in mice was calculated to 1.0–1.3 mg MMH/kg body weight/day (36% increase of lung cancer in males, 33% increase in females) (Toth, 1972), 6.58 mg gyromitrin/kg body weight/day (44% increase of lung cancer in females), 6.58 mg gyromitrin/kg body weight/day (90% increase of cancer in preputial glands in males) (Toth *et al.*, 1981) and 0.8–1.2 mg MFH/kg body weight/day (35% increase of lung cancer in males, 33% increase in females) (Toth and Patil, 1982).

Exposure assessment

In the absence of information on the consumption of False Morel, assumptions are required for the exposure assessment. The exposure assessments in this risk assessment are based on an anticipated consumption twice a month of a meal with 25 g parboiled (boiled twice and boiling water discarded) fresh False Morel, 25 g canned False Morel or 2.5 g dried False Morels on average containing hydrazine/hydrazone derivatives equivalent to 25 mg MMH/kg mushroom. For a consumer weighing 70 kg this intake would correspond to an exposure of 5.95×10^{-4} mg MMH/kg body weight/day. If the dried False Morel is boiled twice and the water discarded both times, the estimated exposure from the dried mushrooms will be lower, but there are apparently no published data on the content of hydrazines in the mushrooms treated this way.

Risk characterization

Teratogenic effects

As the “LOEL” for developmental toxicity after *peroral* application of MMH to rats was determined to be 5.0 mg MMH per kg rat/day, and a human consumer of dried, canned or properly parboiled False Morel (see above) would be exposed to 5.95×10^{-4} mg MMH/kg body weight/day, the calculated margin to humans having the risk of teratogenic effects would be around 800 (assuming humans and rats reacting the same way to MMH).

Carcinogenic effects

Since the metabolism of False Morel hydrazones/hydrazines produces metabolites with a potential to give rise to DNA damage and genotoxic effects, and no threshold has been established for genotoxic carcinogenic substances, the cancer risk can be roughly estimated using a quantitative risk assessment approach. In the approach a linear extrapolation from the high exposure levels in mice to the lower human exposure level is

performed, which requires several assumptions. Thus, in addition to assumptions mentioned above, it is also assumed that man and mouse have the same metabolism and sensitivity, that the survival of mice in the MMH-exposed and control groups are comparable, and that the average weight of a mouse is 25 g and that of man 70 kg. This approach estimates the probability of an increase in human cancer rate, e.g., as extra cancer cases per 10^6 individuals during lifetime exposure. It is then possible to estimate the human cancer risk by relating the outcome of the cancer studies in mice administered MMH in drinking water for lifetime with the estimated amount of MMH consumed by humans through the consumption of False Morel.

In the cancer studies in mice the "LOEL" of MMH (sulfate) after *peroral* application of the compound in drinking water (10 mg/L) corresponds to an exposure of 1.3 mg MMH/kg body weight in males, and 1.0 mg MMH/kg body weight in females. An average consumer being exposed to 5.95×10^{-4} mg MMH/kg body weight/day from parboiled, canned or dried False Morel would have an increased cancer risk of approximately $160\text{--}200 \times 10^{-6}$ based on linear dose-response for cancer induction by MMH. This means that consumers would have an additional lifetime risk of around 160–200 per million of individuals. Overall, a risk of 40–250 extra cancer cases during lifetime per million of Nordic consumers eating the mushroom twice a month (25 g of canned or 25 g of properly pre-treated and cooked or 2.5 g dried False Morel) could be estimated.

Using the "LOEL" from the cancer studies in mice with gyromitrin or with MFH and using the same risk assessment approach as for MMH, gyromitrin would induce 40–80 cancer cases and MFH 170–250 cancer cases per million of individuals during lifetime.

Uncertainties: The quantitative risk assessment suffers from several uncertainties: The use of a linear extrapolation model, the unsatisfactory design of the carcinogenicity studies and the lack of human data, including intake data. Therefore the risk assessment is conservative (worst-case-scenario).

Conclusion: It has been shown that False Morel and its constituents gyromitrin and other hydrazones, as well as the degradation/metabolism products of the hydrazones, N-methyl-N-formylhydrazine (MFH) and monomethylhydrazine (MMH) are carcinogenic in rodents, presumably via a genotoxic mechanism. Therefore, consumption of False Morel may constitute a risk for humans.

Recommendation

False Morel (*Gyromitra esculenta*)¹⁵ should not be consumed as it even after months of drying or after repeated parboiling and discarding of the water, still contains significant amounts of acutely toxic compounds that also are suspected to be genotoxic and carcinogenic.

Listing 4

Database search information

SciFinder by February 2012. Keywords: *Gyromitra*, gyromitrin. PubMed by February 2012. Keywords: Gyromitrin.

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¹⁵ False Morel can legally be sold in Sweden and Finland under specific conditions.

In Sweden, False Morel is not permitted to be sold to the final consumer as fresh or dried mushrooms. However, the preserved mushroom is permitted for sale to the final consumer. For commercial use in restaurants, where adequate knowledge on the preparation of the mushroom is expected to be available, the fresh, dried and preserved False Morel is permitted.

In Finland, the fresh and dried mushroom can be sold to the final consumer with the following, obligatory labelling: False Morel is poisonous. False Morel shall be boiled in water (3 portions of water for 1 portion of mushroom). Dried False Morel shall be soaked in water for at least 2 hours before use (200 g water for 10 g mushroom). After having been soaked, the mushrooms shall be boiled like the fresh mushrooms. When False Morel is dried or boiled good ventilations shall be used. Water used for soaking or boiling shall not be used in foods.

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Hericium coralloides (Scop.) Pers.
(*H. ramosum* (Bull.) Letell.)

Coral Tooth (DK: Koralpigsvamp, FI: Siiliorakas, IS: Broddkórall, NO: Korallpiggsopp, SE: Koralltaggsvamp).

Background and risk assessment

Coral Tooth (*Hericium coralloides*) is an edible mushroom.

It is saprotrophic and possibly parasitic on hardwood stumps and fallen branches, rarely on living hardwood. It is rare in Denmark, Norway, Sweden and Finland and not found in Iceland (Hansen & Knudsen, 1997). It may occasionally be cultivated.

There are no intoxications reported after consumption of Coral Tooth and no natural toxicant constituting a risk for humans has been identified in the mushroom, and there are only few data available on potential bioactive constituents in the mushroom:

Cyathane diterpenoids: Saito *et al.* (1998) have isolated erinacine E, a cyathane xyloside diterpenoid from a culture of Coral Tooth. Erinacine E was shown to be a highly selective binding inhibitor for the kappa opioid receptor in different *in vitro* systems (Kawagishi *et al.*, 1994; 1996a, 1996b). This diterpenoid has also been reported to be a stimulator of nerve growth factor (NGF) synthesis. Anke *et al.* (2002) have isolated

another cyathane xyloside, originally called herical, but later designated erinacine P. Erinacine P was shown to inhibit growth of a large spectrum of fungi and bacteria and to have cytotoxic and hemolytic properties.

It is not known to what extent erinacine E and P (or other cyathane terpenoids) occur in the fruit bodies of Coral Tooth.

The structural formula for erinacine E is shown below.

Erinacine E

Recommendation

Coral Tooth is red-listed in Denmark, Norway and Sweden as near threatened.

It should only be marketed in the Nordic countries, if it is from countries, where it is cultivated or not red-listed.

Listing 1

Database search information

PubMed by December 2012. Keywords: *Herichium*.

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Hericium erinaceus (Bull.) Pers.

Bearded Tooth (Lion's Mane Mushroom, Pom Pom) (DK: Pindsvinepigsvamp ("Pom-Pom"), FI: No Finnish name, IS: Ígulbroddkórall, NO: Piggsvinsopp, SE: Igelkottaggsvamp ("Pom-Pom")).

Background and risk assessment

Bearded Tooth (*Hericium erinaceus*) is an edible mushroom.

It is saprotrophic and possibly parasitic on trunks of hardwood and large diameter branches from living and fallen old deciduous trees, occasionally also on younger damaged growing trees. It is rare in Denmark and Sweden. No data are available for the other Nordic countries (Hansen & Knudsen, 1997).

It is widely cultivated, mainly in Asia (China, Japan, Malaysia and others), not least for being used to promote health and supply medicinal effects. Both fresh and dried fruit bodies are commercialised. When included in products for health-beneficial uses, also mycelial products, fruit body extracts and isolated fractions are used (Kawagishi & Zuang, 2008; Khan *et al.*, 2013; Lindequist *et al.*, 2010; Ma *et al.*, 2010a; Mizuno, 1999).

There are no intoxications reported after consumption of Bearded Tooth. However, due to the extensive use of Bearded Tooth for health-beneficial/medicinal purposes, the mushroom has been thoroughly studied for its content of potentially bioactive constituents. A very

large number of potentially bioactive compounds have been isolated, characterized and investigated for beneficial effects in *in vitro* studies. A few of these compounds have also been tested in experimental animals and in preclinical trials in humans:

Bioactive constituents

Many of the bioactive compounds are *aromatic*, i.e. contains a benzene ring:

Benzyl derivatives: About twenty constituents which can be derived from benzyl alcohol have been isolated from the fruit bodies of the mushroom, with trivial names such as hericenone A, C to L, 3-hydroxyhericenones, erinacerin B, and hericene A to D (Ma *et al.*, 2012; Ueda *et al.*, 2008; Yaoita *et al.*, 2005; Kawagishi *et al.*, 2008; Ma *et al.*, 2010a). Several of the hericenones have in *in vitro* studies been shown to stimulate the synthesis of nerve growth factor (NGF). This has been interpreted as an “anti-dementia” effect, which has some support in experimental studies in rats and preliminary trials in humans (Kawagishi *et al.*, 2008). Other biological effects of certain benzyl derivatives demonstrated *in vitro* include cytotoxic effects (Ma *et al.* 2010b) and protective activity against endoplasmic reticulum stress-dependent cell death (Ueda *et al.*, 2008).

The structural formula for one of these benzyl derivatives, hericene C, is shown below.

Hericene C

Monochlorobenzene compounds: Three 2,5-dimethoxy-monochlorobenzenes were isolated from cultures of *H. erinaceus* (Quian *et al.*, 1990). Two differently substituted 2,5-dimethoxy-chlorobenzenes have been isolated from scrap cultivation beds of Bearded Tooth. *In vitro* these compounds showed protective activity against endoplasmic reticulum stress-dependent cell death (Ueda *et al.*, 2009). It is not known whether these chlorobenzenes are also synthesized in the fruit bodies.

The structural formula for one of these chlorobenzenes, methyl 4-chloro-3,5-dimethoxybenzoate, is shown below.

Methyl 4-chloro-3,5-dimethoxybenzoate

Isoindolinone compounds: At least four different isoindolinone alkaloids have been isolated from the fruit bodies of Bearded Tooth: Hericenone B (isohericenone), erinacerin A, hericerin and a hericerin related compound (without phenylethyl substitution at the nitrogen) (Kawagishi *et al.*, 1990; Kim *et al.*, 2012; Kimura *et al.*, 1991; Kobayashi *et al.*, 2012; Miyazawa *et al.*, 2012; Yaoita *et al.*, 2005; 2012). Only a few studies, all being *in vitro* studies, have explored the potential biological effects of these constituents. Hericenone B showed cytotoxic activity (Kim *et al.*, 2012; Mori *et al.*, 2010), and hericerin inhibitory effects on pollen growth (Kimura *et al.*, 1991).

The structural formula of one of these isoindolinones, hericenone B, is shown below.

Hericenone B

A second group of bioactive constituents in Bearded Tooth containing a number of potentially bioactive constituents are the *diterpenoids*:

Cyathane diterpenoids: More than twenty diterpenoids have been isolated from mycelia of Bearded Tooth. They have all a cyathane skeleton, a tricyclic diterpene structure with an angularly condensed five-, six- and seven-membered ring. Several of these terpenes induce biosynthesis of NGF in cell cultures *in vitro*, e.g. the cyathane terpenoids erinacine A to G (Kawagishi *et al.*, 1994b, 1996a, 1996b). These constituents have been investigated for potential “anti-dementia” effects and to some ex-

tent there are *in vivo* studies in rats and preliminary clinical trials in patients with dementia supporting such an effect (for reviews see Kawagishi & Zhuang, 2007; Ma *et al.*, 2010a). Two of these terpenoids, erinacine A and B, were also demonstrated *in vitro* to reduce activity of methilin-resistant *Staphylococcus aureus* (Kawagishi, 2005).

The structural formula of one of these cyathane derivatives, erinacine E, is shown below.

Erinacine E

Other potentially bioactive constituents in Bearded Tooth are 4-pyranones, statins, ergothioneine and lectins:

4-Pyranones: Two 4-pyranones, 6-methyl-2,5-dihydroxymethyl- and 2-hydroxymethyl-5-hydroxyethyl-4-pyranone, have been isolated from a culture of Bearded Tooth (Quian *et al.*, 1990). Two other 4-pyranones, the dihydropyranones erinapyrone A and B, could also be isolated from cultivated mycelium of the mushroom. The latter two compounds inhibited growth of HeLa cells *in vitro* (Kawagishi *et al.*, 1992; Mizuno, 1999).

The structural formula of one of these pyranones, erinapyrone A, is shown below.

Erinapyrone A

Statins: Small amounts of lovastatin (188 mg/kg; mean of 3 samples) have been identified in cultured mycelia of Bearded Tooth by Chen *et al.* (2012) – for further information on statins, see *Pleurotus ostreatus*.

Ergothioneine: Also ergothioneine 376 mg/kg; (dry weight; mean of 3 samples) have been reported to occur in mycelia of Bearded Tooth (Chen *et al.*, 2012) and in the fruit bodies 960 mg/kg (dry weight; mean of 3 samples) (Lee *et al.*, 2009) – for further information on ergothioneine, see *Lepista nuda*.

Lectins: Kawagishi *et al.* (1994a) have isolated a tetrameric lectin from fresh fruit bodies of Bearded Tooth. The lectin has a molecular mass estimated to 54 kDa with two subunits of 15 kDa and two of 16 kDa. The lectin agglutinates human type A, B and O erythrocytes and pig erythrocytes. The hemagglutinating activity was inhibited by several monosaccharides and sialic acids. The lectin was quite stable at pH 5–10.5 and at temperatures up to 70 °C. At 80 °C only about 25% of the hemagglutinating activity on rabbit erythrocytes remained (Gong *et al.*, 2004). In a study on different lectins from edible mushrooms, the Bearded Tooth lectin, contrary to the other lectins, did not induce mitogenesis (Ho *et al.*, 2004). More recently, another lectin was isolated from the dried fruit bodies of Bearded Tooth. It is a monomer with a molecular mass of 51 kDa. This lectin agglutinates rabbit erythrocytes and this activity is only inhibited by inulin, not by simple sugars. It is stable in the pH range 2–12 and relatively heat-stable up to 70 °C, but loses half of its activity at 80 °C. It is biologically active in different *in vitro* tests. For example, it has been reported to have mitogenic, anti-proliferative and anti-fungal activity (Li *et al.*, 2010). Some lectins in foods, particularly in legumes (and probably also in mushrooms as indicated for *Boletus satanas*) are known to give rise to gastrointestinal distress (Liener, 1983). This is especially the case, if the foods are not sufficiently heat-treated.

Conclusion: Overall, it is not possible to evaluate whether the above-mentioned bioactive compounds in Bearded Tooth may have any health-beneficial or adverse effects in humans consuming this mushroom, as they have mainly been studied *in vitro* and as the quantitative occurrence of these constituents is generally not well described.

Recommendation

Bearded Tooth is red-listed in Denmark, Sweden and Norway as critically endangered.

It should only be marketed in the Nordic countries, if it is from countries where it is cultivated or not red-listed.

Listing 1

Database search information

SciFinder by December 2012. Keywords: *Hericium*. PubMed by December 2012. Keywords: *Hericium*.

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Hericium ramosum (Bull.) Letell.

See *Hericium coralloides*.

Hydnum repandum L.

Wood Hedgehog, ("Pied de Mouton", "Hedgehog") (DK: Almindelig Pigsvamp ("Pigsvamp"), FI: Vaaleaorakas, IS: Gulbroddi, NO: Blek piggsopp, SE: Blek taggsvamp).

Background and risk assessment

Wood Hedgehog (*Hydnum repandum*) is an edible mushroom.

It is terrestrial in both deciduous and coniferous forests. It is common in the Nordic countries up to the subarctic zone (Hansen & Knudsen, 1997).

There are no intoxications reported after consumption of Wood Hedgehog, and no natural toxicants constituting a risk for humans have been identified in the mushroom. However, some biologically active constituents have been identified in Wood Hedgehog:

Bioactive constituents

Polyacetylenic compounds: An acetylenic diepoxide named repandiol has been isolated from the fruit bodies of Wood Hedgehog in an amount corresponding to 150 mg/kg (fresh weight) (Takahishi *et al.*, 1992) or 20 mg/kg (fresh weight) (Millard *et al.*, 2004). The chemical structure was elucidated as (2R,3R,8R,9R)-diepoxy-4,6-decadiyne-1,10-diol and confirmed by synthesis (Takahashi *et al.*, 1992; Millard *et al.*, 2004).

Repandiol was shown to have potent cytotoxic activity against different tumour cells cultivated *in vitro* (Takahashi *et al.*, 1992). Repandiol is a bifunctional alkylating agent. Like for many other bifunctional, alkylating compounds, repandiol alkylates DNA and forms inter-strand cross-links, as demonstrated by *in vitro* incubation of repandiol with restriction fragment of DNA. Such DNA binding and cross-linking activity *in vivo* may result in antitumour and/or carcinogenic activity as demonstrated for many other cross-linking compounds (Rajski & Williams, 1998). However, the cross-linking capability of repandiol was substantially lower than for other, more efficiently cross-linking diepoxyalkanes such as diepoxy-butane and diepoxy-octane, probably due to its reduced rotational flexibility and an increased sterical hindrance (Millard *et al.*, 2004). The structural formula of repandiol is shown below.

Repandiol

Cyathane diterpenoids: Wang *et al.* (2005) have isolated two diterpenoids from fruit bodies of Wood Hedgehog, sarcodonin A and scabronine B with a cyathane skeleton (a fused 5-6-7 tricyclic structure) (Engquist & Stoltz, 2009). It has been demonstrated that scabronine B stimulates the synthesis of nerve growth factor (NFG) in rat cells cultivated *in vitro* (Kita *et al.*, 1998). The structural formula of one of these cyathan diterpenoids, sarcodonin A is shown below.

Sarcodonin A

Ergothioneine: Lee *et al.* (2009) have identified 780 mg/kg (dry weight; mean of tree samples) of ergothioneine in the fruit bodies of Wood Hedgehog. For further information on ergothioneine: see *Lepista nuda*.

Mutagens: Extracts of fruit bodies from Wood Hedgehog were examined for mutagenic activity in a histidine-independent bacterial test system using the *Salmonella typhimurium* strains strain TM677. The mushroom extract was weakly positive. It is plausible that the mutagenic activity was due to repandiol occurring in the mushroom. However, more information is needed to interpret e.g. whether the mutagen is destroyed during the processing of the mushroom for consumption (Grüter *et al.*, 1991).

Overall it is concluded that Wood Hedgehog contains a compound(s) which in *in vitro* studies may damage DNA and be mutagenic. However, there are no *in vivo* studies available on the mushroom or its potential bioactive constituents. Therefore, it is not possible to evaluate whether consumption of Wood Hedgehog may give rise to any adverse effects, in particular after exposure over an extended period of time.

Recommendation

No special recommendation.

Listing 1

Database search information

SciFinder by February 2012. Keywords: *Hydnum*.

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Hydnum rufescens Pers.

Terracotta Hedgehog (DK: Rødgul Pigsvamp ("Pigsvamp"), FI: Rusko-orakas, IS: Rauðbroddi, NO: Rødgul piggsopp, SE: Rødgul taggsvamp).

Background and risk assessment

Terracotta Hedgehog (*Hydnum rufescens*) is an edible mushroom.

It is mycorrhizal with both deciduous and coniferous trees. It is common in the Nordic countries up to the subarctic zone (Hansen & Knudsen, 1997).

There are no intoxications reported after consumption of Terracotta Hedgehog, and no natural toxicant constituting a risk for humans has been identified in the mushroom.

As Terracotta Hedgehog is closely related to Wood Hedgehog (*H. repandum*) and *H. albidum*, which have both been shown to contain constituents that are bioactive in *in vitro* test systems (e.g., cyathane-diterpenoids and an acetylenic diexpoxide), it is plausible that also Terracotta Hedgehog contains these compounds (see *H. repandum*).

Recommendation

No special recommendation.

Listing 1**Database search information**

SciFinder by February 2012. Keywords: *Hydnum*.

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Hygrocybe pratensis (Fr.) Murrill
(*Camarophyllus pratensis* (Fr.) P. Kumm.)

Meadow Waxcap (Buffcap) (DK: Eng-Vokshat, FI: Niittyvahakas, IS: Vallhnúfa, NO: Engvokssopp, SE: Ängsvaxskivling (Ängvaxing)).

Background and risk assessment

Meadow Waxcap (*Hygrocybe pratensis*) is an edible mushroom.

It is probably saprotrophic, growing mostly on unfertilized grassland and pastures. It is common up to the arctic/alpine zones of the Nordic countries, though less common in northern parts of Finland (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Meadow Waxcap, and no natural toxicants constituting a risk for humans have been identified in the mushroom.

Recommendation

No special recommendation.

Listing 2

Database search information

SciFinder by December 2012. Keywords: *Hygrocybe*, *Camarophyllus*.

PubMed by December 2012. Keywords: *Hygrocybe*.

References

Knudsen H & Vesterholt J (2012): Funga Nordica. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. Nordsvamp: 1–1083.

Hygrocybe punicea (Fr.) P. Kumm.

Crimson Waxcap (DK: Skarlagen-Vokshat, FI: Punikkivahakas, IS: Skarlattoppa, NO: Skarlagenvokssopp, SE: Scharlakansvaxskivling).

Background and risk assessment

Crimson Waxcap (*Hygrocybe punicea*) is an edible mushroom.

It is probably saprotrophic and grows on seminatural, high diversity grassland and fixed dunes. It is occasional, but locally common in temperate to subarctic/subalpine zones of the Nordic countries, though with a more southern distribution in Finland (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Crimson Waxcap, and no natural toxicants constituting a risk for humans have been identified in the mushroom.

The colouring matters in yellow and red *Hygrocybe* species are generally, as shown for Crimson Waxcap, anticipated to be related to the pigments secodopas, muscaflavin and betalamic acid in Fly Agaric (*Amanita muscaria*). The pigments are all indicated to be formed enzymatically from L-3,4-dihydroxyphenylalanine (L-DOPA) (Mueller *et al.*, 1997; Steglich & Preuss, 1975; Terradas & Wyler, 1991; von Ardenne *et al.*, 1974). The potential biological effects of these pigments are unknown.

Recommendation

Red-listed as near threatened in Sweden and Denmark. Should only be marketed in the Nordic countries, if it is from countries, where it is not red-listed.

Listing 2

Database search information

SciFinder by December 2012. Keywords: *Hygrocybe*. PubMed by December 2012. Keywords: *Hygrocybe*.

References

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Hygrophorus camarophyllus (Alb. & Schwein.) Dumée, Grandjean & Maire

Arched Woodwax (DK: Sodbrun Sneglehat, FI: Mustavakakas, IS: Sötsniglingur NO: Sotvokssopp, SE: Sotvaxskivling (Sotväxing)).

Background and risk assessment

Arched Woodwax (*Hygrophorus camarophyllus*) is an edible mushroom.

It grows in mycorrhizal association with Pine (*Pinus*) and Spruce (*Picea*). It is very common in hemiboreal and up to subalpine/subarctic zones but very rare in temperate zone (Denmark and the most southern Sweden and Norway) (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Arched Woodwax, and no natural toxicants constituting a risk for humans have been identified in the mushroom.

Recommendation

Arched Woodwax is red-listed in Denmark as critically endangered. Should only be marketed in the Nordic countries, if it is from countries, where it is not red-listed.

Listing 2

Database search information

SciFinder by December 2012. Keywords: *Hygrophorus*. PubMed by December 2012. Keywords: *Hygrophorus*

References

Knudsen H & Vesterholt J (2012): Funga Nordica. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. Nordsvamp: 1–1083.

Hygrophorus hypothejus (Fr.) Fr.

Herald of Winter (DK: Frost-Sneglehat, FI: Hallavahakas, IS: No Icelandic name, NO: Frostvokssopp, SE: Frostvaxskivling (Frostvaxing)).

Background and risk assessment

Herald of Winter (*Hygrophorus hypothejus*) is an edible mushroom.

It forms mycorrhiza in coniferous forests, often along tracks. It is widespread and common in temperate, hemiboreal and boreal areas of the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Herald of Winter, and no natural toxicants constituting a risk for humans have been identified in the mushroom. However, one potential bioactive constituent has been identified in the mushroom. It is a lectin having hemagglutinating activity against human erythrocytes and binding specifically to lactose and N-acetyl-D-galactosamine (Guillot & Coulet, 1974; Veau *et al.*, 1999). The lectin is tetrameric with a molecular mass of 68 kDa with four identical subunits, each with a molecular mass of 17kDa. The lectin shows a sharp fall in hemagglutinating activity when heated to a temperature above 40 °C (Veau *et al.*, 1999). Cooking of the mushroom will probably destroy the lectin's potentially biological effect.

Recommendation

No special recommendation.

Listing 2

Database search information

SciFinder by December 2012. Keywords: *Hygrophorus*. PubMed by December 2012. Keywords: *Hygrophorus*.

References

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Hypholoma capnoides (Fr.) P. Kumm.

Conifer Tuft (DK: Gran-Svovlhat, FI: Kuusilahokka, IS: Viðarnollur (Kirkjugarðsnollur), NO: Svovelsopp, SE: Rökslöjskivling).

Background and risk assessment

Conifer Tuft (*Hypholoma capnoides*) is an edible mushroom.

It is a saprotrophic, mostly fasciculate mushroom found on stumps and roots, of Spruce (*Picea*) and Pine (*Pinus*), mainly in late autumn but also in spring. It is very common in most areas, but rare in subarctic/subalpine and arctic/alpine areas of the Nordic countries (Hansen & Knudsen, 1992; Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Conifer Tuft, and no natural toxicants constituting a risk for humans have been identified in the mushroom.

According to Sterner *et al.* (1982) extracts of Conifer Tuft are mutagenic in the Ames test using the *Salmonella typhimurium* strains TA98, TA100 and TA2637. The extract was found positive in all three strains. The activity was not enhanced in presence of microsomal enzymes. Unfortunately, Sterner *et al* (1982) give no information, whether they had controlled for the potential presence of histidine in the extract. It is well known that false positives may be obtained in the presence of histidine.

Recommendation

Conifer Tuft (*Hypholoma capnoides*) resembles Sulphur Tuft (*Hypholoma fasciculare*) which is poisonous. See Chapter 4.

The mushroom is therefore not regarded as suitable for commercial marketing.

Listing 3

Database search information

SciFinder by February 2012. Keywords: *Hypholoma*.

References

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Hypsizygus marmoreus (Peck.) H.E. Bigelow

Beech Leech (Brown Beech Mushroom, “Brown Shimeji”, White Beech Mushroom, “White Shimeji”) (DK: Bøgehat, FI: Runkoalumuska, IS: No Icelandic name, NO: No Norwegian name, SE: No Swedish name).

Background and risk assessment

Beech Leech (*Hypsizygus marmoreus*) is an edible mushroom.

It is a saprotrophic mushroom, which grows on wood of deciduous trees, especially in Japan and China (Hongo & Izawa, 2006). It is widely cultivated, especially in East Asia, and traded both as a white form, White Beech Mushroom, and as a brown form, Brown Beech Mushroom.

There are no intoxications reported after consumption of Beech Leech, and no natural toxicant constituting a risk for humans has been identified in the mushroom, except for traces of hydrogen cyanide.

Several bioactive constituents with potentially biological effects in humans have been identified in Beech Leech.

Bioactive constituents

Cyanogenic compounds: Only traces of cyanogenic compounds, 0.2 mg per kg of the mushroom have been found in Beech Leech (Shindo *et al.*, 1999). This level of hydrogen cyanide in the fresh mushroom is of no health concern in relation to consumption of Beech Leech.

Statins: High amounts of lovastatin have been found in the mycelia of two forms of Beech Leech, 455 mg/kg dry weight (mean of 35 samples) in a white strain and 424 mg/kg dry weight (mean of 3 samples) in a coloured strain, respectively (Chen *et al.*, 2012). The same investigators reported lower levels, 258 mg/kg dry weight, in fruit bodies of the coloured strain, but gave no information on the white strain. Lo *et al.* (2012), on the other hand, found 628 mg/kg dry weight in the fruit bodies (mean of 3 samples). Lovastatin inhibits the rate limiting enzyme in the production of cholesterol in mammals, including in humans. In rare cases, when statins like lovastatin have been used as drugs, they may give rise to myopathy, characterised by muscular weakness and/or pain and in very rare cases rhabdomyolysis¹⁶ (for further data on statins, see *Pleurotus ostreatus*). These serious adverse effects have not been seen after consumption of Beech Leech.

Ergothioneine: Relatively high amounts of ergothioneine were reported in the mycelia of two forms of Beech Leech, 207 and 221 mg/kg dry weight (for each mean of 3 samples) (Chen *et al.*, 2012). The level was higher in fruit bodies where a normal coloured strain contained 410 mg/kg dry weight. The level in the white strain was not reported. Other investigators have reported much lower levels in dried fruit bodies, 46 mg/kg (mean of 3 samples) (Lo *et al.*, 2012). Ergothioneine in mushrooms has in numerous *in vitro* and in a few *in vivo* studies been shown to have antioxidative and cytoprotective properties against a wide range of cellular stressors and it is anticipated to have a potential as a physiological antioxidant (e.g. Song *et al.*, 2010). For further data on ergothioneine, see *Lepista nuda*.

Ribosome-inactivating protein: A heat tolerant ribosome-inactivating protein, named hypsin, with a molecular mass of 20 kDa, having antifungal and antiproliferative properties, has been isolated from the fruit bodies of Beech Leech (Lam & Ng, 2001).

Polyacetylenic compounds: Three polyacetylenic alcohols have been isolated from liquid mycelium culture of Beech Leech, 2(E)-decene-4,6,8-triyn-1-ol, decene-4,6,8-triyn-1-ol and 8(E)-decene-4,6-diyn-1,2,10-triol. Although the isolated acetylenic constituents were unstable at room temperature, the two triyns were demonstrated to have inhibitory activity against the Oak (*Quercus*) pathogenic fungus *Raffaelea*

¹⁶ Rhabdomyolysis is the breakdown of muscle fibers that leads to the release of muscle fiber contents (myoglobin) into the blood stream. Myoglobin is harmful to the kidney and often causes kidney damage (MedlinePlus Medical Encyclopedia, 2012).

quercivora (Shiona *et al.*, 2013). Due to their instability even at room temperature, it is not likely that these polyacetylenic constituents have any activity in the cooked mushroom.

The structural formula of one of these three acetylenic compounds, 2(E)-decene-4,6,8-triyn-1-ol is show below.

2(E)-Decene-4,6,8-triyn-1-ol

Polyisoprenepolyols: Sawabe *et al.* (1996; 1999) have isolated and elucidated the structure of at least 17 homologous polyisoprenepolyols with 40–70 carbon atoms from the fruit bodies of Beech Leech. The compounds have been name hypsiziprenols. Two of these polyols, hypsiziprenol A₉ and B₁₀ were shown to have inhibitory activities against a strain of *Mycobacterium tuberculosis* (Akihisa *et al.*, 2005). Hypsiziprenol A₉ has also been shown to have antiproliferative activity against human cells cultured *in vitro* (Chan *et al.*, 2004). Whether the two hypsiziprenols have biological effects also *in-vivo* is not known.

The structural formula of one of these polyols, hypsiziprenol B₁₀ is shown below.

Hypsiziprenol B₁₀

Recommendation

No special recommendation.

Listing 1

Database search information

SciFinder by December 2012. Keywords: *Hypsizygyus*.

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Kuehneromyces mutabilis (Schaeff.) Singer & A.H. Sm.
(*Pholiota mutabilis* (Schaeff.) P. Kumm.)

Sheathed Woodtuft (“Two-tone Pholiote”) (DK: Foranderlig Skælhat, FI: Koivunkantosieni, IS: Hverfiskrýfa Hverfisveppur, NO: Stubbeskjell-sopp, SE: Föränderlig tofsskivling).

Background and risk assessment

Sheathed Woodtuft (*Kuehneromyces mutabilis*) is an edible mushroom.

The mushroom is saprotrophic on deciduous wood, rarely on Spruce (*Picea*) from late spring to late autumn. It is very common in most parts of the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Sheathed Woodtuft, and no natural toxicants constituting a risk for humans have been identified in the mushroom.

Recommendation

Sheathed Woodtuft (*Kuehneromyces mutabilis*) resembles Funeral Bell (*Galerina marginata*) which is deadly poisonous. See Chapter 4.

The mushroom is therefore not regarded as suitable for commercial marketing.

Listing 3

Database search information

SciFinder by February 2012. Keywords: *Kuehneromyces*, *Pholiota*.

References

Knudsen H & Vesterholt J (2012): Funga Nordica. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. Nordsvamp: 1–1083.

Laccaria amethystina (Huds.) Cooke

Amethyst Deceiver (DK: Violet Ametysthat, FI: Lehtolohisieni, IS: Fjólu-lakka, NO: Ametystsopp, SE: Ametistskivling).

Background and hazard identification

Amethyst Deceiver (*Laccaria amethystina*) is generally considered edible although some more recent handbooks may warn against the intake due to the high content of arsenic, e.g. Knudsen & Petersen (2009).

It is mycorrhizal with deciduous trees, especially Beech (*Fagus*) but also with coniferous trees. It is very common in Denmark and the southern parts of Norway, Sweden and Finland, and occasional in boreal areas of the same three countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Amethyst Deceiver, but Amethyst Deceiver (and some other *Laccaria* species) bioaccumulate efficiently the toxic element arsenic contrary to other edible mushrooms (Stijve & Bourqui, 1991). Generally, the content of total arsenic is low in mushrooms used as food. The European Food Safety Authority (EFSA) has compiled data on arsenic in 710 samples of various mushroom species from EU countries (EFSA, 2009). The mean level of total arsenic among the mushrooms was in the region of 0.06–0.07 mg/kg fresh weight. The 95th percentile contained about 0.12 mg total arsenic per kg fresh weight.

Exposure/content of arsenic compounds

A series of studies on totally about 80 samples of Amethyst Deceiver, collected in various European countries, show that this species contains 25–250 (mean values 60–180) mg total arsenic per kg dried mushroom (Andersen *et al.*, 1982; Byrne & Tušek-Žnidarič, 1983, Byrne *et al.*, 1979, 1991, 1995; Larsen *et al.*, 1998; Slekovec & Irgolic, 1996; Slekovic *et al.*, 1999; Stijve & Bouqui, 1991; Stijve *et al.*, 1990; Vetter, 2004). This corresponds to approximately 2.5–25 (mean values 6–18) mg per kg fresh Amethyst Deceiver. Amethyst Deceiver growing in arsenic contaminated soils may contain particularly high levels of arsenic. Thus Larsen *et al.* (1998) found a concentration of 1,420 mg per kg dried mushroom, corresponding to approximately 142 mg per kg of fresh weight, in Amethyst Deceiver growing in an area formerly used for wood impregnation with mixtures containing arsenic compounds.

In most mushrooms, arsenic is present in different chemical forms, but is mainly organically bound. The dominant arsenic compound in many mushrooms is arsenobetaine (Slekovec *et al.*, 1997; 1999), a compound of much lower toxicity than most other organic as well as inorganic arsenic compounds as it is not metabolized before excretion by humans (EFSA, 2009). However, in Amethyst Deceiver dimethylarsinic (V) acid (DMA) is the most abundant arsenic species, constituting more than 50% of total arsenic in this mushroom (Byrne *et al.*, 1995; Larsen *et al.*, 1998; Slekovec *et al.*, 1999). Other chemical forms of arsenic occurring in small amounts in Amethyst Deceiver include: methylarsonic acid, trimethylarsine oxide, arsenic acid and traces of arsenobetaine (Larsen *et al.*, 1998; Slekovec *et al.*, 1999).

The structural formula of DMA is shown below:

CH_3
Dimethylarsinic acid (DMA)

Hazard characterisation

The most abundant arsenic compound in Amethyst Deciver, DMA, is used under the name cacodylic acid in pesticides. Cacodylic acid has been investigated for its biological effects in several biochemical/toxicological studies. EFSA's panel on Contaminants in the Food Chain has recently made a risk assessment on arsenic in food (EFSA, 2009). The following text is to a large extent based on this EFSA opinion, focussing on the sections dealing with the biological properties of DMA.

Absorption of organic arsenic compounds is generally greater than 70%. After absorption, DMA is widely distributed to almost all organs and readily crosses the placental barrier. The biotransformation of DMA is not well understood. However it is recognised that arsenic compounds are frequently excreted in urine, often in the form of DMA. Studies in rats have recently demonstrated that DMA may be reduced *in vivo* by a glutathione-dependent enzymatic process to the highly toxic dimethylarsinous acid (Németi & Gregus, 2013). However, the mechanism of this process remains unclear. The EFSA Opinion refers to acute toxicity studies demonstrating oral LD₅₀ values for DMA in rodents: depending on study approximately 1,200 and 1,800 mg/kg body weight (male mouse), 640 mg/kg bw (female rat) and 1,310 and 1,430 mg/kg bw (male rat). In food context, the more relevant repeated dose toxicity studies with DMA indicate organ effects on the urinary bladder, kidneys, and thyroid gland as well as on foetal development. The most sensitive effect is considered to be carcinogenicity of the bladder. No histological alterations were observed in tissues following exposure of rats and mice to high doses of dimethylarsinate (7.8, 94 mg/kg bw/day). DMA is transferred from the mother's circulation through the placenta and crosses the immature blood-brain barrier easily. In developmental toxicity studies with DMA using daily oral (gavage) doses of 0, 4, 12 and 36 mg/kg bw/day in pregnant rat and 0, 3, 12 and 48 mg/kg bw/day in pregnant rabbit during the foetal phase of organogenesis no dose-related effects occurred at exposure levels that were not toxic to the mother. However, maternal and developmental toxicity were observed in the rat at 36 mg/kg bw/day. In the rabbits, at 48 mg/kg bw/day, there was marked maternal toxicity, culminating for most females in abortion and with no surviving foetuses for evaluation. Chronic animal studies with DMA in rats and mice showed no clinical signs of neurotoxicity in rodents at chronic exposure levels of 7.8 and 94 mg/dimethylarsinate/kg bw/day.

An interesting finding is that of Cohen *et al.* (2006; 2007) who demonstrated that DMA (>50mg/L in drinking water) was carcinogenic in rats (urinary bladder) but not in mice. The mechanism for the car-

cinogenic effects of DMA has been extensively investigated but no firm conclusions have been drawn. Although organic arsenic compounds did not induce direct DNA damage at modest concentrations, genetic damage can be induced at high doses and indirectly via oxidative mechanisms. Tokar *et al.* (2012) recently showed that repeated DMA exposure in adulthood promotes tumours or lesions in the kidney and liver initiated by prenatal inorganic arsenic, but acted independently in the urinary bladder, lung and adrenal. Arsenic compounds also cause significant changes in DNA methylation and histone modification, leading to epigenetic silencing or reactivation of gene expression. Genotoxicity experiments *in vitro* and recent animal carcinogenicity studies provide strong support for the suggestion that DMA can act as co-carcinogen in combination with non-metal carcinogens (Yamamoto *et al.*, 1995; Wanibuchi *et al.*, 1996; Nishikawa *et al.*, 2002; Tokar *et al.*, 2012). However, the mechanism for this co-carcinogenic activity is unclear. Overall, arsenic carcinogenesis appears to require the formation of chromosomal damage, and activation of signal transduction pathways promoting survival and expansion of genetically/epigenetically altered cells.

Thus, it is not clear whether DMA is carcinogenic via a genotoxic mechanism, thereby always constituting a risk, or via other mechanisms such as epigenetic, requiring a threshold level to be reached.

Huang *et al.* (2012), in an epidemiological case-control study, recently investigated the correlation between the DNA damage marker 8-hydroxydeoxyguanosine (8-OHdG) in urine or urinary species of arsenic, and the incidence of renal cell carcinoma. Urinary levels of 8-OHdG was significantly related to urinary total arsenic and the odds ratio for renal cell carcinoma had a dose-response relationship. Huang *et al.* (2012) concluded that higher urinary 8-OHdG in arsenic exposed subjects is a strong predictor of renal cell carcinoma.

The EFSA panel on contaminants concluded – although a considerable number of studies have been performed – that there was lack of data on DMA in order to complete a risk characterisation. Furthermore, the Panel identified a need for improved understanding of the human metabolism of organoarsenicals in foods, and the human health implications (EFSA, 2009).

Risk characterization

In the absence of enough data on the mechanism for a potential carcinogenicity risk for humans exposed to DMA, it is concluded that dimethylarsinic acid (DMA) occurring in Amethyst Deceiver cannot be ex-

cluded to be genotoxic and carcinogenic, and accordingly, this mushroom should not be traded as food.

Recommendation

Amethyst Deceiver bioaccumulates efficiently arsenic, particularly in various organic forms, including in specially high amounts dimethylarsinic acid. This compound cannot be excluded to be genotoxic and carcinogenic, and the mushroom should therefore not be traded as food.

Listing 4

Database search information

SciFinder by February 2012. Keywords: *Laccaria* and arsenic/and dimethylarsinic acid/and cacodylic acid/and constituents/and toxicity. Dimethylarsinic acid. PubMed 2009–2012. Keywords: Dimethylarsinic acid.

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Lactarius Pers

Milkcap (DK: Mælkehat; FI: Rousku; IS: Lekta NO: Riske; SE: Riska).

The genus Milkcap (*Lactarius*) comprises 92 species in the Nordic countries. The genus forms mycorrhiza with trees and shrubs in nearly all types of forests, in dunes and alpine habitats. The Milkcap (*Lactarius*) and the Brittlegill (*Russula*) genera are related and belong to the same family, *Russulaceae*. The fruit bodies are characterised by crumbling texture but contrary to the Brittlegill species, the Milkcap species exude a milky juice (latex) after injury (Knudsen & Vesterholt, 2012). Furthermore, a major group of colouring matters, the nitrogen-containing russupteridines in the Brittlegill genus, are not found in the Milkcap genus.

Orange milked species, e.g. Saffron Milkcap (*L. deliciosus*) and False Saffron Milkcap (*L. deterrimus*) and the white milked Fishy Milkcap (*L. volemus*) are considered as edible and good. In Finland, also *L. trivialis*, Rufous Milkcap (*L. rufus*), Woolly Milkcap (*L. torminosus*) and other pungent/acrid tasting Milkcap species are consumed after pre-treatment, especially salting.

The pungent/acrid taste, e.g. of Rufous Milkcap and Woolly Milkcap arises from a few fatty acid esters of the sesquiterpene, velutinal, occurring as an emulsion in specialized *hyphae* of the fruit body. By physical injury of the fruit body by parasites, insects or mammals these tasteless esters are within seconds or minutes converted to pungent, unsaturated sesquiterpene dialdehydes such as velleral, isovelleral and others (Camazine & Lupo, 1984; Daniewski & Vidari, 1999; Favre-Bonvin *et al.*, 1982; Sterner *et al.*, 1983). The pungent dialdehydes have experimentally been shown to produce their irritant effects by interacting with vanilloid receptors on capsaicin-sensitive sensory neurons (Szallasi *et al.*, 1996; 1998). During time, especially after further injury of the fruit body, e.g. pre-treatment before cooking, the pungent sesquiterpenes are further converted by a cascade of enzymatic and chemical reactions, thereby producing a very large number of non-pungent sesquiterpenes, e.g., a series of furanoid sesquiterpenes of which some are artefacts and some like the dihydroxyfuran shown below are true natural metabolites (Sterner *et al.*, 1988). Each Milkcap species produces a characteristic pattern of such compounds. These reactions may change the colour and taste of the milky juice (Ayer & Browne, 1981; Clericuzio *et al.*, 2008; Sterner & Anke, 1995; Sterner *et al.*, 1988). Insufficiently pre-treated and/or cooked, pungent Milkcap species, e.g. Rufous Milkcap, have given rise to mild gastrointestinal intoxications (Barceloux, 2008; Benjamin, 1995; Bresinsky & Besl, 1985; Flammer & Horak, 2003; Roth *et al.*, 1990).

The structural formulae of the precursor ester stearylvelutinal, two of the pungent dialdehydes, velleral and isovelleral and one non-pungent metabolite, a dihydroxyfuran sesquiterpene, are shown below.

Stearylvelutinal

Velleral

Isovelleral

Dihydroxyfuran sesquiterpene derivative

Many of these sesquiterpene metabolites are biologically active in experimental studies, where they have shown irritant, antifeedant, cytotoxic, antifungal, insecticidal and mutagenic activities (Clericuzio *et al.*, 2008; Daniewski & Vidari, 1999; Daneiowski *et al.*, 1995; Jonassohn *et al.*, 1997; Szallasi *et al.*, 1996). Especially, the antifeedant properties have been demonstrated, and it is believed that the sesquiterpene metabolites constitute a sort of defence system for the Milkcap (Anke & Sterner, 1991; Anke *et al.*, 1989; Camazine & Lupo, 1984; Camazine *et al.*, 1983; Daniewski *et al.*, 1995; Sterner *et al.*, 1985a, b; Vidari & Vita-Finzi, 1995).

Database search information

SciFinder by September 2011. Keywords: *Lactarius*. PubMed: 2011-August 2012. Keywords: *Lactarius*.

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Lactarius deliciosus (L.) Gray

Saffron Milkcap (DK: Velsmagende Mælkehat, FI: Männynleppärousku, IS: Matglætingur Ljúflekta, NO: Furumatriske, SE: Läcker riska (Läcker-riska, Tallblodriskä)).

Background and risk assessment

Saffron Milkcap (*Lactarius deliciosus*) is an appreciated, edible mushroom.

It forms mycorrhiza with Pine (*Pinus*). It is common in the temperate and hemiboreal zones and occasional in boreal-subalpine/subarctic zones in the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Saffron Milkcap. However, some constituents with potentially bioactive activity have been reported.

Bioactive constituents

Sesquiterpenes: Contrary to most species of Milkcap (*Lactarius*) no volatile esters have been detected in Saffron Milkcap. The only sesquiterpenes detected in the intact mushroom are two different orange-yellow guaiane esters having the same azulene alcohol moiety (Bergendorff & Sterner, 1988; Clericuzio *et al.*, 2008; Daniewski & Vidari, 1999). By injury or during ageing, these esters are hydrolysed, forming a series of blue, violet and red sesquiterpenes having an azulene structure (Clericuzio *et*

al., 2008; Daniewski & Vidari, 1999; Liu, 2007). The biological activity of one of the guaiane esters and the mushroom metabolites deterrol (an alcohol) and its corresponding aldehyde lactaroviolin has been studied. The three sesquiterpenes are weakly mutagenic in the Ames test, deterrol and lactaroviolin are moderately cytotoxic, and lactaroviolin also exhibits weakly antibacterial activity in *in vitro* studies (Anke *et al.*, 1989). The green colour formed after physical injury or with ageing emerges from a mixture of the orange-yellow coloured precursors and the violet-blue metabolites lactaroviolin and deterrol (Sternner & Anke, 1995). The redbrown discolouration of urine, which can be seen after consumption of Saffron Milkcap (and the related False Saffron Milkcap (*L. deterrimus*)) are caused by metabolites of the azulene pigments and are considered to be of no safety concern (Bresinsky & Besl, 1985; Kunzfeld, 1953).

The structural formulae of the two guaiane metabolites, lactaroviolin and deterrol are shown below.

Lactaroviolin

Deterrol

Indole compounds: Muszynska *et al.* (2011; 2012) isolated the indole compounds 5-hydroxytryptophan, serotonin and melatonin, as well as the tryptophan metabolite kynurenine sulfate from fruit bodies of Saffron Milkcap. It was shown that boiling of Saffron Milkcap significantly changed the content of these indoles. 5-Hydroxytryptophan and serotonin were completely destroyed, and tryptophan, 5-methyltryptophan and tryptamine had been formed in amounts of 88, 38 and 32 mg/kg (dry weight) corresponding to approximately 9, 4 and 3 mg/kg of fresh mushroom, respectively. Some of these compounds may act as tissue hormones and neurotransmitters, which in higher amounts may damage the nervous systems (Stone *et al.*, 2003).

Mutagens: Sterner *et al.* (1982) tested an extract of Saffron Milkcap in the Ames test using the *Salmonella typhimurium* strains TA98, TA100 and TA2637. The extract was positive in all three strains. The activity was not enhanced in the presence of microsomal enzymes. Unfortunately, Sterner *et al.* (1982) give no information, whether they had controlled for the potential presence of histidine in the extract. It is well known that false positives may be obtained in the presence of histidine. Also Morales *et al.* (1990a, 1990b) studied mushroom extracts in the Ames test. Extracts of freshly harvested and frozen Saffron Milkcap mushrooms were reported to be weakly mutagenic in the *Salmonella* strains TA98 and TA100 (for the fresh mushroom less than doubling in mutation frequency). Extracts of canned Saffron Milkcap were negative both in the Ames test and in the Chinese Hamster Ovary (CHO/HPRT) cell mutation assay. Apparently, Morales *et al.* (1990a, 1990b) did not control for possible histidine in the extracts for the Ames test. Thus, there is no strong indication that extracts of the Saffron Milkcap are mutagenic. However, one of the constituents, a sesquiterpene stearate and two metabolites of this ester, are weakly mutagenic in the Ames test (see above).

Lectins: One dimeric lectin with a molecular weight of about 37kDa (subunits with molecular weights of about 19 and 18 kDa) has been isolated from Saffron Milkcap and characterised (Guillot *et al.*, 1991). Some lectins, especially those that are thermostabile, may give rise to gastrointestinal discomfort (Liener, 1983), but the lectin in Saffron Milkcap has not been studied for biological activity or thermostability.

Recommendation

No special recommendation.

Listing 2

Database search information

SciFinder by September 2011. Keywords: *Lactarius*. PubMed: 2011–August 2012. Keywords: *Lactarius deliciosus*.

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Lactarius deterrimus Gröger

False Saffron Milkcap (DK: Gran-Mælkehat, FI: Kuusenleppärousku, IS: Matlekta, NO: Granmatriske, SE: Blodrisk (Granblodrisk)).

Background and risk assessment

False Saffron Milkcap (*Lactarius deterrimus*) is an appreciated, edible mushroom.

It forms mycorrhiza with Spruce (*Picea*), often with young stands. It is very common in temperate-boreal zones and rare, but locally occasional, in subalpine/subarctic zones in the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of False Saffron Milkcap. However, some potentially bioactive constituents have been reported.

Bioactive constituents

Sesquiterpenes: Contrary to most species of Milkcap (*Lactarius*) no volatile esters have been detected in False Saffron Milkcap. The only sesquiterpenes detected in the intact mushroom are two orange-yellow guaiane esters having the same azulene alcohol moiety (Bergendorff & Sterner, 1988; Clericuzio *et al.*, 2008; Daniewski & Vidari, 1999). By injury or during ageing, these esters are hydrolysed, forming a series of blue, violet and red sesquiterpenes with azulene structure (Clericuzio *et al.*,

2008; Daniewski & Vidari, 1999; Liu, 2007). The biological activity of the guaiane stearate esters and the mushroom metabolites deterrol (an alcohol) and its corresponding aldehyde lactaroviolin, has been studied. The three sesquiterpenes are weakly mutagenic in the Ames test, deterrol and lactaroviolin are moderately cytotoxic, and lactaroviolin also exhibits weak antibacterial activity in *in vitro* studies (Anke *et al.*, 1989). The green colour formed after physical injury of the mushroom or with ageing emerges from a mixture of the orange-yellow coloured precursors and the violet-blue metabolites lactaroviolin and deterrol (Sterner & Anke, 1995). The redbrown discolouration of urine, which can be seen after consumption of False Saffron Milkcap (and the related Saffron Milkcap (*L. deliciosus*)) is caused by metabolites of the azulene pigments and are considered to be of no safety concern (Bresinsky & Besl, 1985; Kunzfeld, 1953).

The structural formulae of the metabolites lactaroviolin and deterrol are shown below.

Lactaroviolin

Deterrol

Mutagens: Sterner *et al.* (1982) tested an extract of False Saffron Milkcap in the Ames test using the *Salmonella typhimurium* strains TA98, TA100 and TA2637. The extract was found positive in all three strains. The activity was not enhanced in presence of microsomal enzymes. Unfortunately, Sterner *et al.* (1982) give no information, whether they had controlled for the potential presence of histidine in the extract. It is well known that false positives may be obtained in the presence of histidine. In the same study, the pungent sesquiterpene velleral was negative, whereas the pungent isovelleral was positive in two of the *Salmonella* strains (TA100 and TA2637). However, extracts of False Saffron Milkcap were also weakly positive in a histidine-independent bacterial strain. More information is needed to interpret e.g. whether the mutagen is destroyed during the processing of the mushroom for consumption (Grüter *et al.*, 1991).

Indole compounds: Muszynska *et al.* (2007) isolated a series of simple biologically active indole compounds including tryptophan, tryptamine and melatonin. Some of these constituents are tissue hormones and neurotransmitters and high intakes of tryptophan can damage the nervous system (Stone *et al.*, 2003).

Lectins: One dimeric lectin with a molecular weight of about 31 kDa with two identical subunits has been isolated from False Saffron Milkcap and characterised (Giollant *et al.*, 1993). Some lectins, especially thermostable ones may give rise to gastrointestinal discomfort (Liener, 1983), but the lectin in the False Saffron Milkcap has not been studied for biological activity or thermostability.

Recommendation

No special recommendation.

Listing 2

Database search information

SciFinder by September 2011. Keywords: *Lactarius*. PubMed: 2011–August 2012. Keywords: *Lactarius deterrimus*.

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Lactarius necator (Bull.) Pers.
(*L. plumbeus* s. auct., *L. turpis* (Weinm.) Fr.)

Ugly Milkcap (DK: Olivenbrun Mælkehat, Manddraber-Mælkehat, FI: Mustarousku, IS: Grænlekta, NO: Svartriske, SE: Svartriska).

Background and risk assessment

Ugly Milkcap (*Lactarius necator*)¹⁷ is no longer considered as an edible mushroom.

It grows mycorrhizal with Spruce (*Picea*) and Birch (*Betula*). It is very common in temperate-hemiboreal zones in the Nordic countries, except in Iceland, where it is very rare (Knudsen & Vesterholt, 2012).

It has given rise to several cases of gastroenteritis, probably due to insufficient pre-treatment, e.g. salting and/or parboiling (Barceloux, 2008; Benjamin, 1995; Bresinsky & Besl, 1985; Roth *et al.*, 1990).

Ugly Milkcap contains several bioactive constituents:

¹⁷ Index Fungorum/Species Fungorum (2012) uses *L. turpis* as current name, but Knudsen & Vesterholt (2012), ArtDatabanken (2011) and Danish Mycological Society (2012) use *L. necator* as preferred name.

Bioactive constituents

Sesquiterpenes: Ugly Milkcap contains stearoylvelutinal and 6-ketostearoylvelutinal which both by physical injury of the fruit body are degraded/metabolised to pungent unsaturated dialdehyde sesquiterpenes such as velleral and *epi*-piperdial as well as to non-pungent sesquiterpenes (Sternner, 1989; Sternner & Anke, 1995; Daniewski & Vidari, 1999). Contrary to other *Lactarius* species, where the velutinal esters are completely converted within few minutes after injury, the main part of the esters in the Ugly Milkcap remains unchanged even several hours after injury (Sternner, 1989).

The structural formulae of the two velutinal esters and the two pungent dialdehydes velleral and *epi*-piperdial are shown below.

epi-Piperdial

Beyond the pungent sesquiterpenes, formed by injury, the fruit bodies of Ugly Milkcap contain a highly mutagenic alkaloidal pigment:

Mutagens: In 1982 Knuutinen and von Wright reported that an extract of Ugly Milkcap showed mutagenic activity in the Ames test, using the histidine requiring strains TA98 and TA100 of *Salmonella typhimurium* to detect the mutations. The investigators analysed for histidine in the mushroom extract and concluded that the levels of histidine did not influence the revertant frequencies in the assay (Knuutinen & von Wright, 1982). Subsequently von Wright *et al.* (1982) confirmed the finding with an extended set of *Salmonella* strains TA1535, TA1537 and TA1538. The extract was positive regarding induction of mutations in *Salmonella* strains TA98, TA100, TA1535 and TA1537, but not in TA1538. Supplying the *Salmonella* assay with a metabolizing system from rat livers did not increase the mutation frequency. In this study, extracts of several *Lactarius* and other mushroom species, were tested in the Ames assay. The Ugly Milkcap extract was by far the most mutagenic mushroom extract in the study (Knuutinen & von Wright, 1982; von Wright *et al.*, 1982). There was no indication that the investigators had controlled for the presence of histidine in the mushroom extracts in this second study.

Also Sterner *et al.* (1982a) have tested extracts of the Ugly Milkcap in the Ames test using *Salmonella* strains TA98, TA100 and TA2637 as indicators of mutagenic activity. The extract was positive in the TA100 and TA2637 strains. The same investigators also compared the mutagenic activity of extracts of a Finnish pickled commercial Ugly Milkcap product with that of an extract of fresh Ugly Milkcap (Sterner *et al.*, 1982b) in *Salmonella typhimurium* strains TA98, TA2637 and TA100. The comparison was done without including a metabolic activation system in the assay and there was no indication that the potential presence of histidine in the extract had been controlled for. Both extracts showed mutagenic activity, particularly in *Salmonella* strain TA2637. The high muta-

genic activity of extracts of Ugly Milkcap was confirmed by Grüter *et al.* (1991) using a histidine-independent bacterial test system.

Alkaloidal pigments: Suortti and colleagues reported in 1983–1984 about the isolation, characterization and quantification of a highly mutagenic principle in Ugly Milkcap (Suortti, 1984a; 1984b; Suortti & von Wright, 1983; Suortti *et al.*, 1983; von Wright & Suortti, 1983a, 1983b). The mutagen was isolated as bright red crystals with a molecular weight of 264 and was tentatively identified as the alkaloid 7-hydroxycoumaro[5,6-*c*]cinnoline, which was given the trivial name necatorin. Necatorin was shown to occur in amounts of 3–20 mg per kg fresh mushroom. Pure necatorin was shown to be highly mutagenic in the Ames test in all three of the *Salmonella* strains TA98, TA100 and TA1537. Addition of a metabolizing system (S9 fraction) reduced the mutagenic activity (von Wright & Suortti, 1983a, b). The strong mutagenic activity of necatorin in the Ames test has later been confirmed (Hilger *et al.*, 1985). However, necatorin was negative in the host-mediated assay and in the micronucleus test (von Wright & Suortti, 1983a, b).

Boiling/blanching destroys about half of the necatorin in the mushroom leaving about 25% of the original content in the mushroom and about 25% in the water (Suoritti, 1984a, 1984b).

Subsequently, Fugmann *et al.* (1984) studied the pigments in Ugly Milkcap and isolated a pigment with the same molecular weight as the pigment, necatorin, isolated and tentatively identified as 7-hydroxycoumaro[5,6-*c*]cinnoline by Suoritti *et al.* (1983). Fugmann *et al.* (1984) allocated the structure 5,10-dihydroxy-dibenzo[de,h][1,6]naphthyridin-6-one to the alkaloidal pigment and named it necatorone. The structure of necatorone was confirmed by total synthesis (Hilger *et al.*, 1985). Direct comparison of necatorin, provided by Suortti, with necatorone by chromatographic and spectroscopic methods established that the two compounds were identical (Hilger *et al.*, 1985).

Later, two other alkaloidal pigments, 4,4'-binecatorone and 10-deoxy-4,4'-binecatorone, were isolated from Ugly Milkcap. There are no biological data available on these two alkaloids (Klamann *et al.*, 1989).

The structure of necatorone (necatorin) and the two binecatorones are shown below.

Necatorin

Necatorone

4,4'-Binecatorone

10-Deoxy-4,4'-binecatorone

Overall, it is concluded, that Ugly Milkcap contains an alkaloidal pigment, necatorone (necatorin), which is highly mutagenic in the Ames test, and that pickling or boiling/blanching the mushroom only partly reduces its mutagenicity.

Recommendation

Ugly Milkcap contains the heat-stable necatorone (necatorin), which is suspected to be genotoxic. Therefore, Ugly Milkcap should not be used in commercial trade.

Listing 4

Database search information

SciFinder by September 2011. Keywords: *Lactarius*. PubMed: 2011–August 2012. Keywords: *Lactarius necator*, *Lactarius plumbeus*, *Lactarius turpis*.

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Lactarius plumbeus s. auct.

See *Lactarius necator*.

Lactarius rufus (Scop.) Fr.

Rufous Milkcap, (Red Hot Milkcap) (DK: Rødbrun Mælkehat, FI: Kangasrousku, IS: No Icelandic name, NO: Rødbrun pepperriske, SE: Pepparriska).

Background and risk assessment

Rufous Milkcap (*Lactarius rufus*) is, after appropriate pre-treatment, an edible mushroom.

It forms mycorrhiza with conifers, especially Spruce (*Picea*) and Pine (*Pinus*), but also with deciduous trees. It is very common in the Nordic countries in temperate to subarctic/subalpine zones, but rare in alpine/arctic zones (Knudsen & Vesterholt, 2012).

Consumption of Rufous Milkcap has given rise to several cases of gastroenteritis, probably due to insufficient pre-treatment, e.g. salting and/or parboiling (Barceloux, 2008; Benjamin, 1995; Bresinsky & Besl, 1985; Flammer & Horak, 2003; Roth *et al.*, 1990). Rufous Milkcap contains several potentially bioactive constituents:

Bioactive constituents

Sesquiterpenes: Rufous Milkcap contains stearoylvelutinal and 6-ketostearoylvelutinal (Daniewski & Vidary, 1999), which on physical injury of the fruit bodies (e.g. when cleaned and cut into pieces for food

use) are both degraded within seconds or a few minutes to sesquiterpenes, including the pungent, unsaturated dialdehydes isovelleral and velleral (Sterner, 1989; Daniewski & Vidary, 1999). By salting, pickling or parboiling, the pungent and gastroirritating isovelleral and velleral are further degraded to other sesquiterpenes, thereby abolishing the pungent and irritating activity.

The structural formulae of the two velutinal esters and the two dialdehydes are shown below:

Stearoylvelutinal

6-Ketostearoyl velutinal

Velleral

Isovelleral

Lectins: A lectin with six identical subunits has been characterized in Rufous Milkcap. Each subunit has a molecular weight of about 17 kDa. The lectin is quite stable, but 85% of the activity is lost by heating the mushroom at 65 °C (Panchak & Antoniuk, 2007). Some lectins in foods, particularly in legumes (and possibly in mushrooms) are known to give rise to gastrointestinal distress (Liener, 1983), especially if the food is not sufficiently heat treated.

Muscarine: Rufous Milkcap has also been shown to contain trace amounts (totally less than 2 mg/kg fresh weight) of the toxicants L-(+)-muscarine (approximately 40%) and its stereoisomers (approximately 60%) (Stadelmann *et al.*, 1976). However, such low amounts of L-(+)-muscarine and its stereoisomers are of no toxicological concern at normal consumption levels of Rufous Milkcap (Bresinsky & Besl, 1985; Stadelmann *et al.*, 1976). The structural formula of L-(+)-muscarine is shown below:

L-(+)-Muscarine

Mutagens: In 1982 Knuutinen and von Wright reported that extracts of Rufous Milkcap are weakly mutagenic in the Ames assay, using the histidine-requiring *Salmonella typhimurium* strains TA98 and TA100 to detect the activity. The finding was confirmed in studies with the *Salmonella* strains TA1535, TA1537 and TA1538 (von Wright *et al.*, 1982). As the test system detects back-mutations from histidine requirement to histidine auxotrophy, presence of histidine or precursors of this amino acid (which may occur in mushrooms) in the test solution has the capability

to induce false positives. It is unclear whether von Wright *et al.* (1982) controlled for the presence of histidine or histidine precursors in the mushroom extract.

Also Sterner *et al.* (1982) tested Rufous Milkcap extracts for mutagenic activity in the Ames test, using the *Salmonella* strains TA98, TA100 and TA2637 as indicators of mutagenic activity. The extract was reported to be positive in all three strains but inclusion of a metabolizing system from rat liver (S9) did not enhance the mutagenic activity. However, Sterner *et al.* (1982) gave no information whether they controlled for the potential presence of histidine in the extract and thereby induced false positives. In the same study, the pungent sesquiterpene velleral was negative whereas isovelleral was positive in two of the three *Salmonella* strains (TA100 and TA2637). However, isovelleral is likely to be destroyed during cooking. When Grüter *et al.* (1991) studied extracts of Rufous Milkcap in a histidine-independent bacterial test system, they could also demonstrate a weakly positive activity. Overall, it is likely that thoroughly cooked Rufous Milkcap has lost its mutagenic activity.

Recommendation

Before consumption, Rufous Milkcap needs to be pre-treated (salted/heat-treated) to destroy the acrid substances in the mushroom and water from pre-treatment should be discarded.

Listing 2

Database search information

SciFinder by September 2011. Keywords: *Lactarius*. PubMed 2011 – August 2012. Keywords: *Lactarius rufus*.

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Lactarius torminosus (Schaeff.) Gray

Woolly Milkcap (DK: Skægget Mælkehat, FI: Karvarousku, IS: Loðlekta Loðglætingur, NO: Skjeggriske, SE: Skäggriska).

Background and risk assessment

Woolly Milkcap (*Lactarius torminosus*) is, after appropriate pre-treatment, an edible mushroom.

It forms mycorrhiza with Birch (*Betula*) in forests, parks and gardens, in arctic habitats with Dwarf Birch (*Betula nana*). It is very common in the Nordic countries (Knudsen & Vesterholt, 2012).

Woolly Milkcap is like certain other pungent species of Milkcap (*Lactarius*), such as Rufous Milkcap (*L. rufus*) consumed especially in central and eastern parts of Europe. Occasionally, the consumption gives rise to gastroenteritis, probably due to insufficiently pre-treated mushrooms (Barceloux, 2008; Benjamin, 1995; Bresinsky & Besl, 1985; Flammer & Horak, 2003; Roth *et al.*, 1990). Some potentially bioactive constituents have been identified in Woolly Milkcap:

Bioactive constituents

Sesquiterpenes: As other pungent species of Milkcap, Woolly Milkcap contains stearylvelutinal (Daniewski & Vidary, 1999). Upon physical injury stearylvelutinal is within seconds or minutes converted chemi-

cally or enzymatically to the pungent sesquiterpenes piperdial and velleral (Sterner *et al.*, 1985). According to Daniewsky & Vidari (1999) also isovelleral has been detected in Woolly Milkcap. The pungent sesquiterpenes may be further degraded, for example by pre-treatment of the mushroom before cooking.

The structural formulae of stearoylvelutinal and the pungent velleral are shown below:

Mutagens: In 1982 Knuutinen and von Wright reported that Woolly Milkcap extracts are weakly mutagenic as detected by the histidine-requiring *Salmonella typhimurium* strains TA 98 and TA 100 in the Ames assay. Subsequently, von Wright *et al.* (1982) confirmed the finding in three other indicator strains of *Salmonella typhimurium*, TA1535, TA1537 and TA1538.

Further confirmation on mutagenic activity in extracts of Woolly Milkcap was obtained by Sterner *et al.* (1982) who also used the Ames test with the *Salmonella* strains TA98, TA100 and TA2637 as indicators. The extract was positive in all three strains but was not enhanced when including a metabolizing system (S9 mix). Sterner *et al.* (1982) gave no information whether they controlled for the potential presence of histidine in the extract tested. The presence of histidine could have resulted in false positives. In the same study, the pungent sesquiterpene velleral

was negative, whereas the pungent isovelleral was positive in two of the three *Salmonella* strains (TA100 and TA2637). However, isovelleral is likely to be destroyed during cooking.

Ergothioneine: Lee *et al.* (2009) have identified 820 mg/kg (dry weight; mean of tree samples) of ergothioneine in the fruit bodies of Woolly Milkcap. For further information on ergothioneine, see *Lepista nuda*.

Recommendation

Before consumption, Woolly Milkcap needs to be pre-treated (salted/heat treated) to destroy the acrid substances in the mushroom and water from pre-treatment should be discarded.

Listing 2

Database search information

SciFinder by September 2011. Keywords: *Lactarius*. PubMed: 2011–August 2012. Keywords: *Lactarius torminosus*.

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Lactarius trivialis (Fr.) Fr. (*L. utilis* (Weinm.) Fr.)

No UK name (DK: Nordisk Mælkehat (Slimet Mælkehat), FI: Haaparousku, Kalvashaaparousku, IS: No Icelandic name, NO: Hulriske (Blek hulriske), SE: Skogsriskä).

Background and risk assessment

*Lactarius trivialis*¹⁸ is, after appropriate pre-treatment, an appreciated, edible mushroom.

It forms mycorrhiza with Birch (*Betula*) and Spruce (*Picea*). It is occasional in the temperate zone, although rare in Denmark. Otherwise it is very common in the Nordic countries (Knudsen & Vesterholt, 2012).

No intoxications have been reported after consumption of *L. trivialis*. However, some bioactive constituents have been identified in this mushroom.

¹⁸ *Lactarius utilis* is considered as a separate species in the lists of commercial mushrooms in Finland, but it is considered as a synonym for *L. trivialis* by ArtDatabanken (2011), Danish Mycological Society (2012), Knudsen & Vesterholt (2012) and Index Fungorum/Species Fungorum (2012).

Bioactive constituents

Sesquiterpenes: *L. trivialis* contains both stearylvelutinal and 6-ketostearylvelutinal (Daniewski & Vidary, 1999). Upon injury of the mushroom fruit body, these compounds within a very short time are degraded chemically or enzymatically to pungent, unstable sesquiterpenes, which may be further degraded during pre-treatment and cooking of the mushroom.

The structural formulae of stearylvelutinal and the pungent velleral are shown below:

Stearylvelutinal

Velleral

Muscarine: Trace amounts (totally less than 2 mg/kg fresh weight) of the toxicants L-(+)-muscarine (approximately 40%) and its stereoisomers (approximately 60%) have been detected in *L. trivialis* (Stadelmann *et al.*, 1976). However, such low amounts of L-(+)-muscarine and its stereoisomers are of no toxicological concern at normal consumption levels of *L. trivialis* (Bresinsky & Besl, 1985; Stadelmann *et al.*, 1976). The structural formula of L-(+)-muscarine is shown below:

L-(+)-Muscarine

Mutagens: Sterner *et al.* (1982) tested an extract of *L. trivialis* in the Ames test using the *Salmonella typhimurium* strains TA98, TA100 and TA2637. The extract was found positive in all three strains. The activity was not enhanced in the presence of a metabolizing system prepared from rat liver (S9 mix). Unfortunately, Sterner *et al.* (1982) gave no information whether they had controlled for the potential presence of histidine in the extract. It is well known that false positives may be obtained in the presence of histidine.

Recommendation

Before consumption: *Lactarius trivialis* needs to be pre-treated (salted/heat-treated) to destroy the acrid substances in the mushroom and water from pre-treatment should be discarded.

The mushroom is red-listed as near threatened in Denmark. Should only be marketed in the Nordic countries, if it is from countries, where it is not red-listed.

Listing 2

Database search information

SciFinder by September 2011. Keywords: *Lactarius*. PubMed: 2011–August 2012. Keywords: *Lactarius trivialis*, *Lactarius utilis*.

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Sterner O, Bergman R, Kesler E, Magnusson L, Nilsson B, Wickberg B, Zimerson E & Zetterberg G (1982): Mutagens in larger fungi I. Forty-eight species screened for mutagenic activity in the *Salmonella*/microsome assay. *Mutation Research* 101: 269–281.

Lactarius turpis (Weinm.) Fr.

See *Lactarius necator*.

Lactarius utilis (Weinm.) Fr.

See *Lactarius trivialis*.

Lactarius volemus (Fr.) Fr.

Fishy Milkcap (DK: Spiselig Mælkehat, FI: Kultarousku, IS: No Icelandic name, NO: Mandelriske, SE: Mandelriskä).

Background and risk assessment

Fishy Milkcap (*Lactarius volemus*) is an appreciated, edible mushroom.

It forms mycorrhiza in deciduous and coniferous forests. It is rare in temperate, hemiboreal and southern parts of boreal zones of the Nordic countries (Knudsen and Vesterholt, 2012).

Like all Milkcap (*Lactarius*) species, Fishy Milkcap exudes latex. The content of rubber (a relatively low molecular weight rubber composed of 160–300 *cis*-isoprene units) is very high in Fishy Milkcap, compared to other Milkcap species and, in fact, to other fungal species, as it is up to 7 g per kg fresh weight of the mushroom (Anderson *et al.*, 1961; Stewart *et al.*, 1965; Tanaka *et al.*, 1994). It is not known whether the high content of *cis*-polyisoprene may influence the digestibility of Fishy Milkcap.

There are no intoxications reported after consumption of Fishy Milkcap, and no natural toxicant constituting a risk for humans has been identified in the mushroom.

Recommendation

Fishy Milkcap is red-listed in Denmark. Should only be marketed in the Nordic countries, if it is from countries, where it is not red-listed.

Listing 2

Database search information

SciFinder by September 2011. Keywords: *Lactarius*. PubMed: 2011–August 2012. Keywords: *Lactarius volemus*.

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Leccinum albstipitatum den Bakker & Noordel.

See *Leccinum aurantiacum*.

Leccinum aurantiacum s. lato
(*L. albstipitatum* den Bakker & Noordel.,
L. quercinum (Pilát) E.E. Green & Watling)

Orange Bolete (Orange Aspen Bolete, Orange Oak Bolete) (DK: Orange Aspe-Rørhat, Rustrød Ege-Rørhat, FI: Haavanpunikkitatti, IS: Asparlubbi, NO: Ospeskrubb, SE: Aspsopp).

Background and risk assessment

Orange Bolete (*Leccinum aurantiacum*)¹⁹ is edible and good after thorough cooking.

It is mycorrhizal with Poplar (*Populus*), Oak (*Quercus*), Birch (*Betula*) and rarely with Willow (*Salix*), Lime (*Tilia*) and Beech (*Fagus*), in subalpine and arctic/alpine zones also with Bearberry (*Arctostaphylos*). It is occasional in Denmark and common in temperate-subalpine areas of the Nordic countries (Knudsen & Vesterholt, 2012).

¹⁹ Index Fungorum/Species Fungorum (2012) and Knudsen & Vesterholt (2012) have *L. albstipitatum* as preferred name but ArtDatabanken (2011) and Danish Mycological Society (2012) use *L. aurantiacum* s. lato as preferred name. Danish Mycological Society (2012) and Knudsen & Vesterholt (2012) include *L. quercinum* in *L. aurantiacum*.

No toxicants have been identified in Orange Bolete. However, there are many reports on mild intoxications with gastrointestinal symptoms after consumption of different *Leccinum* species (e.g., Beug 2007; 2009; 2010; 2011; 2012; Beug *et al.*, 2006; Mattilsynet, 2012). *Leccinum* species have to be thoroughly cooked in order to destroy the unknown but rather heat-stable toxicant(s) giving rise to these symptoms.

Several phenolic and other hydroxylated pigments have been identified in this mushroom (Bresinsky *et al.*, 1974; Massow & Huber, 1977). However, no toxicological data were available on these potentially bioactive constituents.

Muszynska *et al.* (2011) have identified several indole compounds in Orange Bolete, but at low levels corresponding to approximately 1 mg/kg (fresh weight) or lower, except for serotonin found at a level of approximately 32 mg/kg (fresh weight). However, serotonin is apparently completely destroyed during cooking (Muszynska *et al.*, 2012).

Recommendation

Thorough heat-treatment is necessary as insufficiently cooked *Leccinum* mushrooms can give rise to intoxications.

Listing 2

Database search information

SciFinder by January 2012. Keywords: *Leccinum*.

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Leccinum quercinum (Pilát) E.E. Green & Watling)

See *Leccinum aurantiacum*.

Leccinum scabrum (Bull.) Gray

See *Leccinum* species, other.

Leccinum species, other, e.g. *L. scabrum* (Bull.) Gray

Other *Leccinum* species, e.g. Brown Birch Bolete (DK: Andre skælrørhatte fx Brun Birke-Rørhat, FI: Muut *Leccinum* lajin tatit, IS: Aðrir lubbar-kúalubbi, NO: Andre skrubbar arter – Brunskrubbar, SE: Andra soppar av släktet *Leccinum*-Björksopp).

Background and risk assessment

Leccinum species growing in the Nordic countries e.g. Brown Birch Bolete (*L. scabrum*) are all edible after thorough cooking, but of varying culinary quality.

They are mycorrhizal, especially with Birch (*Betula*), but also with Poplar (*Populus*), Oak (*Quercus*), Hornbeam (*Carpinus*), Hazel (*Corylus*), Beech (*Fagus*) and some coniferous trees such as Pine (*Pinus*) and Spruce (*Picea*), often in moist habitats (Knudsen & Vesterholt, 2012). Brown Birch Bolete is very common in the temperate-subalpine zone and occasional in arctic/alpine zone of the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Brown Birch Bolete. However, other *Leccinum* species, and especially Orange Birch Bolete (*Leccinum versipelle*), have given rise to many intoxications with gastrointestinal symptoms due to insufficient heat-treatment (e.g.,

Beug 2007; 2009; 2010; 2011; 2012; Beug *et al.*, 2006; Mattilsynet, 2012). Generally, *Leccinum* species have to be thoroughly cooked in order to destroy the unknown but rather heat-stable toxicant(s) giving rise to these symptoms.

Polyphenolic constituents with anticipated bioactive properties have been identified in Brown Birch Bolete, e.g. 3,4,5-hydroxybenzaldehyde (Edwards & Elseworthy, 1967) as well as phenolic and other hydroxylated pigments (Besl & Bresinsky, 1977).

Recommendation

Thorough heat treatment of *Leccinum* species is necessary as consumption of insufficiently cooked *Leccinum* species may give intoxications.

Listing 2

Database search information

SciFinder by January 2012. Keywords: *Leccinum*.

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Leccinum versipelle (Fr. & Hök) Snell

Orange Birch Bolete (DK: Rød Birke-Rørhat, FI: Koivunpunikkittatti, IS: Reyðilubbi (Rauðhetta), NO: Rødskrubb, SE: Tegelsopp (Tegelröd björksopp)).

Background and risk assessment

Orange Birch Bolete (*Leccinum versipelle*) is edible and good when sufficiently cooked.

It grows mycorrhizal with Birch (*Betula*) and especially with Dwarf Birch (*Betula nana*) in arctic/alpine zone. It is common in Denmark and southern parts of Norway and Sweden, very common in hemiboreal-arctic/alpine zones, but rare in Iceland (Knudsen & Vesterholt, 2012).

Consumption of Orange Birch Bolete has given rise to several intoxications with gastrointestinal symptoms. This mushroom needs very thorough cooking, in order to destroy some relatively heat-stable toxicant(s) of unknown chemical structure (e.g., Beug 2007; 2009; 2010; 2011; 2012; Beug *et al.*, 2006; Matttilsynet, 2011).

Nieminen and co-workers (2006) have published a study on the potential myo- and hepatotoxic effects of different wild mushrooms in mice. The animals (six females per group) were for 5 days supplied large quantities of dried and powdered wild mushrooms in the feed. One of

the mushrooms, Orange Birch Boletus, was given as 1.8%, 3.6% or 5.4% of the feed. The feeding resulted in exposures corresponding to 19, 36 or 53 g per kg body weight of the mice per day, respectively, corresponding to 1.3, 2.5 or 3.2 kg mushroom per person (70 kg) per day of fresh mushroom. All mice were healthy at the end of the study. Of the many clinical-chemical parameters studied LDL cholesterol was significantly increased in a dose dependent manner. Creatine kinase was significantly increased at the two highest dose levels. No histological pathological changes in studied organs were found. Whether the observed increased creatine kinase (indicator of myopathy) in mice eating very high amounts of Orange Birch Boletus may be relevant for humans eating very large amount of the mushroom is difficult to evaluate.

Recommendation

Thorough heat treatment is necessary as consumption of insufficiently cooked Orange Birch Boletus may give intoxications.

Listing 2

Database search information

SciFinder by January 2012. Keywords: *Leccinum*.

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Leccinum vulpinum Watling

Foxy Bolete (DK: Fyrre-Rørhat, FI: Männynpunikkitatti, IS: -lubbi, NO: Furuskrub, SE: Tallsopp (Rävsopp)).

Background and risk assessment

Foxy Bolete (*Leccinum vulpinum*) is edible and good if sufficiently cooked.

It grows mycorrhizal with species of *Pinaceae* and Bearberry (*Arctostaphylos*). It is occasional in Denmark and in southern temperate parts of Norway and Sweden and common in boreal regions of the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Foxy Bolete, and no natural toxicant constituting a risk for humans has been identified in the mushroom.

However, other *Leccinum* species, and especially Orange Birch Bolete, have given rise to many intoxications with gastrointestinal symptoms due to insufficient heat treatment (e.g., Beug 2007; 2009; 2010; 2011; 2012; Beug *et al.*, 2006; Mattilsynet, 2012). Generally, *Leccinum* species have to be thoroughly cooked in order to destroy the unknown but rather heat-stable toxicant(s) giving rise to these symptoms.

Recommendation

Thorough heat treatment is necessary as insufficiently cooked Foxy Bolete can give intoxications.

Listing 2

Database search information

SciFinder by January 2012. Keywords: *Leccinum*.

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Lentinula edodes (Berk.) Pegler (*L. edodes* (Berk.)
Singer)

Shiitake (DK: Shiitake, FI: Siitake, IS: Shii-take sveppur, Tókasveppur, NO: Shiitake, SE: Shiitake (Ekmussling, Ekskivling)).

Background and risk assessment

Shiitake (*Lentinula edodes*) is an appreciated edible mushroom.

It is saprotrophic on branches, logs and trunks in deciduous forests with Oak (*Quercus*) and is native, especially in Japan and China (Hongo & Izawa, 2006).

Shiitake is, next to Button Mushroom, the most cultivated and consumed mushroom in the world. In 1997, the year from which the latest world production figures were found, it was cultivated in 1.6 million tons (Chang & Miles, 2004). However, more recently Chang & Wasser (2012) reported that the total world production of cultivated mushrooms in 2009 has been estimated to be 24 million tons, of which more than 20 million tons have been produced in China. Approximately one quarter of this production comprises Shiitake. It was originally cultivated in East Asia (Japan, China and Korea), but is now also cultivated in e.g. Europe and the United States. Shiitake is commercially available as fresh, dried, canned or otherwise processed. Especially in Asia it is also widely

used to promote health and supply medicinal effects. When included in products for health-beneficial uses also extracts and isolated fractions are used (Bisen *et al.*, 2010; ; Chang, 1996; Chang & Wasser, 2012; Lindequist *et al.*, 2010; Mizuno *et al.*, 1995).

Several intoxications have been reported after consumption of Shiitake, apparently in almost all cases after consumption of raw or insufficiently cooked mushrooms. The toxicant is supposed to be the thermolabile polysaccharide lentinan but the mushroom is also reported to contain other bioactive constituents.

Bioactive constituents

Lentinan: Consumption of Shiitake (*Lentinula edodes*) may result in a toxic or hypersensitivity reaction manifested as flagellate dermatitis (linear groups of erythematous papules, most frequently on the trunk) (Adler and Larsen, 2011; Adriano *et al.*, 2013; Auth *et al.*, 2005; Carlson *et al.*, 2010; Chu *et al.*, 2013; Diaz-Corpas *et al.*, 2011; Garg & Cockayne, 2008; Hanada & Hashimoto, 1998; Hyry & Kariniemi, 1998; Haas *et al.*, 2001; Kopp *et al.*, 2009; Hérault *et al.*, 2010; Lapresta *et al.*, 2011; Lippert *et al.*, 2003; López-Núñez *et al.*, 2011; Maier & Herzinger, 2007; Mak & Wakelin, 2006; Nakamura, 1977, 1992; Nakamura & Kobayashi, 1985; Poppe *et al.*, 2012; Ricar *et al.*, 2012; Reefman *et al.*, 2013; Schön, 2005; Soo *et al.*, 2006; Tarvainen *et al.*, 1991; Wagner and Sachse, 2011). In Japan, where most of these cases have appeared, it is quite common to consume the mushroom raw or half-cooked. The dermatitis has been described both after consumption of raw and half-cooked Shiitake, but apparently not after consumption of the thoroughly cooked mushroom.

Patch testing and skin prick test are inappropriate as this is not an allergic phenomenon. The exact pathogenesis of the dermatitis is unknown. However, it is speculated that the cause is a toxic reaction to lentinan, a thermolabile polysaccharide in Shiitake. Lentinan is indicated to have anti-tumour properties and the polysaccharide is used as adjuvant in cancer therapy in Japan. Some patients receiving lentinan have also developed a condition similar to flagellate dermatitis (Hanada & Hashimoto, 1997).

The view that consumption of Shiitake may give health benefits to consumers is particularly strong in some areas of Asia. In this context a study on cholesterol-lowering effects of Shiitake consumption showed that 17 of 49 patients withdraw from the study because of rash or abdominal discomfort, and two had marked eosinophilia. One of the latter patients was subsequently challenged for 14 days with Shiitake and eosinophilia appeared again. Levy *et al.* (1998) studied 10 healthy subjects

ingesting 4 g Shiitake powder for 10 weeks (study A) and later for 3–6 month (study B). Four of the subjects in each study (three being the same in both studies) responded with increased blood eosinophils, serum major basic protein, stool eosinophil-derived neurotoxin, and factors that enhanced eosinophil viability. Anti-Shiitake IgE was not found, and IgG was increased in two subjects. Gastrointestinal symptoms coincided with eosinophilia in two subjects. Stopping exposure resulted in symptoms disappearing. Thus, daily ingestion of Shiitake powder in five of 10 healthy persons provoked non-IgE-mediated systemic modulation of eosinophils (Levy *et al.*, 1998).

Another type of hypersensitivity effect, an allergic reaction, to Shiitake was described by Goikoetxea *et al.* (2009) in a patient that showed oesophageal symptoms after mushroom ingestion. The allergenicity was confirmed by skin prick tests, basophile activation test, and histamine release test.

Formaldehyde: Formaldehyde occurs naturally in Shiitake (Mason *et al.*, 2004). It is an essential metabolic intermediate in the biosynthesis of purines, thymidine and certain amino acids, and it is also produced via metabolism of some amino acids and xenobiotics. The generation of endogenous formaldehyde involves the two enzymes gamma-glutamyl transpeptidase and L-cysteine sulfoxide lyase (Liu *et al.*, 2013). The background endogenous blood concentration is approximately 2–3 mg/liter in humans, monkeys and rats (Casanova *et al.*, 1988; Heck *et al.*, 1985).

Formaldehyde has been reported in concentrations of 3–23 mg/kg (IARC, 1982), 1–90 mg/kg (IPCS, 1989), 0.01–98 mg/kg (Clayes *et al.*, 2009) and 1–140 mg/kg (Yau, 2007) in a variety of foods. The significance of ingestion of food as a source of formaldehyde exposure for the general population is questionable. Shiitake may contain higher amounts of formaldehyde than other foods. Mason *et al.* (2004) in a first part of a study reported levels ranging from 110–240 mg/kg (fresh weight, 7 samples) and in the second part of the study 100–320 mg/kg (fresh weight, 6 samples). In the second part of the study it was ensured that the mushroom had not been exposed to formaldehyde at any step of the production or later. The authors concluded that fresh Shiitake contains measurable concentrations in the region of 100–300 mg/kg (fresh weight), and that this formaldehyde is of natural origin in the mushroom. They also concluded that some, but not all of this formaldehyde may be derived from thermal, acidic or enzymatic breakdown of larger endogenous molecules in the mushroom during the analytical procedure. Storage at 4 °C for 10 days did not change the concentration, whereas frying in oil for 6 minutes reduced the content of formaldehyde

significantly (Mason *et al.*, 2004). Other studies have confirmed the presence of formaldehyde in Shiitake. Yamazaki *et al.* (1980) reported levels in the range 25–143 mg/kg (fresh weight, 46 samples) with no significant change of original content of formaldehyde after drying, but with a significant (less than 100%) increase after acidic or enzymatic treatment of the fresh or dried mushroom. Yau (2007) reported 6–54 mg formaldehyde/kg in fresh Shiitake and 100–406 mg/kg in dried mushrooms (dry weight), and Liu *et al.* (2005) 119–494 mg/kg in dried mushrooms containing 11–13% residual water.

The International Agency for Research on Cancer (IARC, 2006) has evaluated formaldehyde for carcinogenetic activity. They found that there is sufficient evidence for formaldehyde being carcinogenic in humans and in experimental animals. The conclusion that formaldehyde is carcinogenic in humans is mainly based on epidemiological studies suggesting a causal relationship between inhalation exposure to formaldehyde and cancer in humans. Support is obtained from animal carcinogenicity studies and mechanistic studies indicating that both genotoxicity and cytotoxicity play important roles in the carcinogenesis of formaldehyde in nasal tissues.

In the Twelfth Report on Carcinogens (NTP, 2011a, 2011b) formaldehyde is listed as “known to be a human carcinogen,” virtually based on the same data as considered by IARC (2006). However, in the background document for development of the “WHO Guidelines for Drinking-water Quality,” it is stated that “There is little evidence that formaldehyde is carcinogenic by the oral route” (WHO, 2005). Similarly, the “European Food Safety Authority’s Scientific Panel on food additives, flavourings, processing aids and materials in contact with food” has especially considered the safety in use of formaldehyde as a preservative during the manufacture of food additives. In its opinion the Panel concluded that there is no evidence indicating that formaldehyde is carcinogenic by the oral route (EFSA, 2006).

An exposure assessment under a worst case scenario characterized by consumption of 100 g fresh Shiitake with a formaldehyde content of 320 mg/kg fresh mushroom, once a week all the year, would result in an average daily intake of 0.08 mg formaldehyde/kg body weight, assuming a person weighing 60 kg. This is a lower exposure than the Tolerable Daily Intake (TDI) calculated for formaldehyde (0.15 mg/kg body weight) by the World Health Organisation for drinking water (WHO, 1996), and also lower than the oral Reference Dose (RfD) of 0.2 mg formaldehyde/kg body weight/day set by the United States Environmental Protection Agency (IRIS, 1990). Both these values are based on a No

Observed Adverse Effect Level (NOAEL) of 15 mg/kg body weight/day determined in a two-year oral toxicity study in rats (Til *et al.*, 1989).

The structural formula for formaldehyde is shown below.

Formaldehyde

Statins: There is very different information on the occurrence of statins in fresh fruit bodies of Shiitake. Whereas Lo *et al.* (2012) reported relatively high amounts of lovastatin (317 mg/kg, mean of 3 samples), Lin *et al.* (2013) only found small amounts (2.7 mg/kg, 3 samples), Chen *et al.* (2012) at a level of 412 mg/kg (mean of 3 samples), and Chen *et al.* (2012) no lovastatin at all. For further information on statins, see *Pleurotus ostreatus*.

Ergothioneine: Lee *et al.* (2009) reported fresh fruit bodies of Shiitake to contain ergothioneine at a level of 1,860 mg/kg (mean of 3 samples), Chen *et al.* (2012) at a level of 412 mg/kg (mean of 3 samples), Nguyen *et al.* (2012) at a level of 123 mg/kg (mean of 3 samples) and Lo *et al.* (2012) at a level of 12 mg/kg (mean of 3 samples), whereas Ey *et al.* (2007) reported levels below the limit of detection. Dubost *et al.* (2006) reported a content of 2,090 mg/kg (mean of 4 samples) in dried Shiitake, which corresponds to approximately 200 mg/kg fresh mushroom. When grown in submerged culture Shiitake mycelia produced from 600 to 3,500 mg ergothioneine/kg of mycelia (dry weight), the quantity depending on the composition of the growth medium (Tepwong *et al.*, 2012). For further information on ergothioneine, see *Lepista nuda*.

Agaritine: Agaritine has been detected in fresh Shiitake at the low level of 0.82 mg/kg (Hashida *et al.*, 1990), but could not be demonstrated in dried Shiitake (limit of detection 300 mg/kg, dry weight, corresponding to less than approximately 30 mg/kg fresh weight) (Stijve & Pittet, 2000) and canned Shiitake (Andersson *et al.*, 1999). For further information on agaritine, see Button Mushroom (*Agaricus bisporus*).

Cyanogenic compounds: Stijve & de Meijer (1999) have detected small amounts of cyanogenic compounds in fresh Shiitake, corresponding to 6.5–12 mg/kg (duplicate analysis), whereas Shindo *et al.* (1999) were unable to detect any hydrogen cyanide in the two samples studied. It is estimated that the content of hydrogen cyanide in cooked Shiitake would be so low, that it is of no health concern (Stijve & de Meijer, 1999).

Polyisoprenoid alcohols: Shiitake contains approximately 20 mg/kg (fresh weight) of polyisoprenoid alcohols having 15–19 isoprene units (dolichols) with dolichol-17 dominating. Additionally, α -unsaturated polyisoprenoid alcohols with corresponding chain lengths have also been detected. It is suggested that these polyisoprenoid alcohols, at adequate quantities, can modulate membrane properties (Wojtas *et al.*, 2004).

Acetylenic compounds: The acetylenic compound octa-2,3-diene-5,7-diyne-1-ol (named lentinamycin) has been isolated from the culture broth of Shiitake. Lentinamycin has been demonstrated to have antimicrobial effects against gram-negative bacteria (Komemushi *et al.*, 1996). It is not known, whether lentinamycin is also produced in fruit bodies of Shiitake.

Lectins: A number of carbohydrate-binding lectins have been isolated from Shiitake mycelia and the broth in which the mushroom was cultivated (Tsivileva *et al.*, 2001; Vetchinkina *et al.*, 2008a; 2008b; 2008c). However, no data were given on the thermal and pH stability of the lectins.

A lectin with a molecular mass of 43 kDa has been isolated from Shiitake fruit bodies. It had hemagglutinating activity, which was demonstrated with rabbit erythrocytes. This activity was specifically inhibited by N-acetylgalactosamine, N-acetylglucosamine and mannose. No information was given on the stability of the lectin at various pH conditions and at various temperatures. Eghianruwa *et al.* (2011) isolated a homotetrameric lectin with a molecular mass of 71 kDa from Shiitake fruit bodies, with subunits of approximately 18 kDa. The lectin binds non-specifically to N-acetylglucosamine. The hemagglutinating activity is stable up to 70 °C but the activity is completely abolished at 90 °C. The lectin is unchanged at pH 2–11 (Jeune *et al.*, 1990; Wang *et al.*, 1999).

Some lectins in foods are known to give rise to gastrointestinal distress, if the food is not sufficiently cooked (Liener, 1983). Whether any of the lectins in Shiitake could give rise to adverse effects after consumption of the mushroom is not known.

Purine alkaloids: Three adenine derivatives 4-(6-amino-9H-purin-9-yl)-2R, 3R-dihydroxybutyric acid (eritadenine), deoxyeritadenine and 4-(6-amino-9H-purin-9-yl)-propionic acid have been isolated from Shiitake (Chibata *et al.*, 1969; Kamiya *et al.*, 1969, 1972; Saito *et al.*, 1970). As Shiitake mushrooms have been shown to have cholesterol reducing effects in both rats (Kaneda & Tokuda, 1966; Yagishita *et al.*, 1978; Yoon *et al.*, 2011) and humans (Suzuki *et al.*, 1976), and eritadenine has been found to have plasma cholesterol reducing effects in rats (e.g. Chibata *et al.*, 1969; Rokujo *et al.*, 1970; Takashima *et al.*, 1974), considerable interest has been devoted to these compounds. Eritadenine has been reported to occur at fairly high levels in Shiitake – in the range 500–700

mg/kg in the cap and in the range 300–400 mg/kg in the stem (dry weight) (Saito *et al.*, 1975; Vitanyi *et al.*, 1998). In more recent studies, up to ten times higher levels have been reported in Shiitake fruit bodies, about 3,200 to 6,300 mg/kg dry weight (Enman *et al.*, 2007), corresponding to approximately 320–630 mg/kg fruit body (fresh weight). In submerged culture, eritadenine was detected at similar levels in mycelia and broth (Enman *et al.*, 2008). The level in the mycelia was in the range 160–1100 mg/kg (dried biomass) (Enman *et al.*, 2008; Lelik *et al.*, 1997). Whether eritadenine could have any adverse effects in humans is not known.

The structural formula for eritadenine is shown below:

Eritadenine

Sulfur-containing volatile constituents: Fresh fruit bodies of Shiitake are almost odourless (Wu & Wang, 2000). However, after cutting/crushing, drying and then soaking and heating some 20 sulfur-containing, organic, volatile substances may be formed (Chen & Ho, 1986; Chiron & Michelot, 2005; Morita & Kobayashi, 1967). Fresh Shiitake contains a tetrathio-dipeptide, lentinic acid, which after sequential enzymatic reactions by γ -glutamyl transpeptidase and C/S-lyase, and subsequent thermochemical reactions may give rise to several sulfur-containing flavouring substances (Höfle *et al.*, 1976; Sneed *et al.*, 2004). According to Chen & Ho (1986) the quantitatively most important sulfur-containing compounds in fresh homogenates of Shiitake fruit bodies are 1,2,4-trithiolane, 1,2,4,5-tetrathiane and lenthionine (1,2,3,5,6-pentathiepane), occurring at levels of approximately 3, 9 and 9 mg/kg, respectively.

The major volatile flavouring substances identified in Shiitake have been risk assessed by the European Food Safety Authority (EFSA, 2012). They were found to be of no safety concern at estimated levels of intake. However, most of the sulfur-containing volatiles could not be predicted to be metabolized to innocuous products. Therefore animal studies were considered and No Observed Adverse Effect Levels (NOAEL's) were de-

terminated in order to establish safety margins for the food use (EFSA 2012). Lenthionine, the characteristic flavouring substance in Shiitake, is reported at a level of approximately 9 mg/kg of fresh, homogenised Shiitake fruit bodies (Chen & Ho, 1986). With a human intake of 100 g Shiitake once a week for life-time, an average daily intake of 0.02 mg lenthionine/kg body weight/day would result, assuming a body weight of 60 kg. When comparing this estimated level of intake with the NOAELs estimated in the 5 animal studies (0.2–7 mg/kg body weight/day) used for the evaluation of these flavourings by EFSA (2012), a safety margin between 100 and 3,500 was established. For other major cyclic sulfur-containing flavourings the safety margins would be the same or higher.

The structural formula for lenthionine is shown below:

Lenthionine

Toxicity studies

Mutagenicity studies: von Wright *et al.* (1982) reported that extracts of Shiitake are weakly mutagenic in the Ames assay, using the histidine-requiring *Salmonella typhimurium* strains TA1535, TA1537 and TA1538. As the test system detects back-mutations from histidine requirement to histidine auxotrophy, presence of histidine or precursors of this amino acid (which may occur in mushrooms) in the test solution has the capability to induce false positives. It is unclear whether von Wright *et al.* (1982) controlled for the presence of histidine or histidine precursors in the mushroom extract. However, Grüter *et al.* (1991), using a histidine-independent bacterial test system, identified no mutagenic activity in extracts of Shiitake. Altogether, it is not likely that extracts of Shiitake are mutagenic.

Short-term oral mouse study: Nieminen and co-workers (2009) have published a study on the potential myo- and hepatotoxic effects of different cultivated mushrooms in mice. The animals (six females per group) were for 5 days supplied large quantities of dried and powdered cultivated mushrooms in the feed. One of the cultivated mushrooms, Shiitake, was given as 1.8%, 3.6% or 5.4% of the feed. The feeding resulted in mice being exposed to 19, 42 and 61 g/kg body weight per day, respectively, which is equivalent to humans ingesting 1.4, 2.9 and 4.3 kg

Shiitake per person (70 kg), or when corrected for body surface constant, 113, 244 and 358 g fresh Shiitake per person (70 kg) per day, respectively. Of the many clinical parameters studied, plasma bilirubin and creatine kinase levels were significantly increased, but not in a dose-related manner. Plasma HDL/total cholesterol was significantly reduced, reaching statistical significance at the two highest doses of Shiitake intake (Nieminen *et al.*, 2009). The HDL/total cholesterol lowering effect might be due to the mushroom containing eritadenine.

Oral 28-day rat study: Two groups, each of 10 male and 10 female rats, received by gavage either an aqueous suspension of 2 g/kg body weight/day of an extract of cultivated Shiitake mycelium or distilled water (control group) for 28 days. Body weight and food consumption were significantly reduced in the group administered Shiitake extract, especially in males, but the weight reduction was less than 10% at the end of the study, when comparing with the control group. Of the many clinical-biochemical parameters measured serum total cholesterol was significantly reduced in dosed animals. No toxicologically relevant effect was observed on hematological and biochemical examinations, absolute and relative organ weights, macroscopic findings and histological findings at the end of the administration (Yoshioka *et al.*, 2010).

Recommendation

Shiitake should be thoroughly cooked, as consumption of the raw or insufficiently cooked mushroom may cause dermatitis.

Listing 1

Database search information

SciFinder by January 2012. Keywords: *Lentinula* or *Lentinus* or Shiitake.
PubMed by December 2012. Keywords: *Lentinula* or *Lentinus* or Shiitake.

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Lentinus edodes (Berk.) Singer

See *Lentinula edodes*.

Lepiota olivieri Barla

See *Chlorophyllum olivieri*.

Lepiota rhacodes (Vittad.) Quél.

See *Chlorophyllum rachodes*.

Lepista nebularis (Batsch) Harmaja

See *Clitocybe nebularis*.

Lepista nuda (Bull.) Cooke
(*Tricholoma nudum* (Bull.) P. Kumm.)

Wood Blewit (DK: Violet Hekseringshat ("Blåfod"), FI: Sinivalmuska, IS: Fjólujússa, NO: Blå ridderhat, SE: Blåmusseron).

Background and risk assessment

Wood Blewit (*Lepista nuda*) is an edible mushroom. However, Wood Blewit may give rise to gastrointestinal symptoms, sometimes severe, if eaten raw – or insufficiently cooked (Beug *et al.*, 2006; Bresinsky & Besl, 1985; Roth *et al.*, 1990).

Wood Blewit is a saprotrophic mushroom. It grows in gardens, parks and forests in nitrogen rich soil, e.g. deep needle beds, heaps of leaves and compost, often in fairy rings. It is very common in temperate/hemiboreal/boreal zones and occasional in subalpine/subarctic zones of the Nordic countries (Knudsen & Vesterholt, 2012). Wood Blewit may also be cultivated.

No intoxications have been reported after consumption of thoroughly cooked Wood Blewit. However, it contains several potentially bioactive constituents.

Bioactive constituents

Hydrogen cyanide: Wood Blewit may contain small amounts of cyanogenic compounds corresponding to 15–29 mg hydrogen cyanide per kg fresh mushroom (Stijve & de Meijer, 1999). During cooking, most of the hydrogen cyanide disappears and the precursors are apparently destroyed. Therefore, consumption of cooked Wood Blewit does not pose any hazard with respect to hydrogen cyanide.

Lectins: Like many other mushrooms the Wood Blewit contains lectin(s). Zaremba (1973) isolated and partially purified a lectin from Wood Blewit and demonstrated that it has hemagglutinating activity on different animal erythrocytes. Also Mikiashvili *et al.* (2006) have demonstrated lectin activity in extracts from fruit bodies of the mushroom (on rabbit erythrocytes). However, the lectin(s) is(are) not well characterised, neither with respect to binding capacity and stability nor to molecular weight.

Ergothioneine: Wood Blewit may contain 5.5 g per kg (dry weight) in the fruit bodies of the non-protein amino acid ergothioneine as shown by Lee *et al.* (2009). Ergothioneine is biosynthesized by mycobacteria, cyanobacteria and non-yeast-like fungi, including many edible mushrooms. For instance, dried fruit bodies of edible *Pleurotus* species, Shiitake (*Lentinula edodes*) and Button Mushroom (*Agaricus bisporus*) may contain in the range of 0.1–2.9 g ergothioneine per kg (Chen *et al.*, 2012a; Dubost *et al.*, 2006; Ey *et al.*, 2007; Lee *et al.*, 2009; Lin *et al.*, 2013; Lo *et al.*, 2012). Generally ergothioneine has been found in somewhat lower amounts in other edible mushrooms (Chen *et al.*, 2012a; Dubost *et al.*, 2006; Ey *et al.*, 2007; Lee *et al.*, 2009; Lin *et al.*, 2013; Lo *et al.*, 2012). Whereas food processing destroys some bioactive constituents, a considerable proportion of the ergothioneine remains unchanged (Ulziijargal *et al.*, 2013).

Ergothioneine has in numerous *in vitro* and in a few *in vivo* studies been shown to have antioxidative and cytoprotective properties against a wide range of cellular stressors and it is anticipated to have a potential as a physiological antioxidant (e.g. Song *et al.*, 2010). It has been shown that ergothioneine can be absorbed from food and accumulate in specific tissues of mammals (including humans) via the highly specific ergothioneine transporter (Cheah and Halliwell, 2012; Gründemann, 2012). However, the physiological role of ergothioneine is not well understood and the consequence of ergothioneine deficiency is unclear. Ergothioneine transporter has been suggested as a possible factor in the autoimmune diseases rheumatoid arthritis and Crohn's disease and patients with rheumatoid arthritis have high levels of ergothioneine (Cheah & Halliwell, 2012; Ey *et al.*, 2007; Gründemann, 2012; Seebeck, 2013;

Weigand-Heller *et al.*, 2012). No adverse effects have been related to even high intakes of the compound. The structural formula of ergothioneine is shown below.

Ergothioneine

Tryptophan metabolites: Fruit bodies of Wood Blewit have been shown to contain several metabolites of tryptophan: 3-indole acetic acid, 3-indole propionic acid, kynurenine and 3-hydroxykynurenine (Kohlmunzer & Wegiel, 2001). As no quantitative data were available, it is not possible to estimate the potential risk from these bioactive constituents.

Acetylenic compounds: Two acetylenic compounds, diatretyne 2 and 3, have been isolated from the culture liquid of Wood Blewit and their structures elucidated as (2E)-7-cyano-2-heptene-4,6-diyynoic acid and (2E)-10-hydroxy-2-decene-4,6,8-triynoic acid, respectively (Anchel, 1953; 1955; 1958; 1959; Anchel *et al.*, 1962; Heatley & Stephenson, 1957; Silverman & Anchel, 1958). Diatretyne 2 was studied for its antibiotic properties and was shown to inhibit the growth of several different bacteria and fungi (Anchel, 1958). More recently, Dighe & Agathe (2000) demonstrated the presence of an antibacterial polyacetylenic compound in a culture filtrate from Wood Blewit. The compound was not well characterized, but as it was water soluble, it is unlikely to be one of the more lipophilic diatrynes. It is not known whether the above-mentioned acetylenic compounds also can be found in the fruit bodies.

The structural formula for one of these diatretynes, diatretyne 2 is shown below.

(2E)-7-Cyano-2-heptene-4,6-diyynoic acid (Diatretyne 2)

Other metabolites: Extracts from the fruit body or mycelia from Wood Blewit have been shown to have antimicrobial activity, e.g. against certain human pathogenic bacteria and certain plant pathogenic fungi (Chen *et al.*, 2012a; Chen & Huang, 2009). Chen *et al.* (2012b) isolated and identified three bioactive constituents inhibiting the zoospore germination of *Phytophthora capsici* from the fluid of Wood Blewit grown in culture: 2-methoxy-5-methyl-6-methoxy-p-benzoquinone, 6-hydroxy-2H-pyran-3-carbaldehyde and indole-3-carbaldehyde. There are no data available demonstrating the potential occurrence of these bioactive compounds in the fruit bodies of Wood Blewit.

Recommendation

No special recommendation.

Listing 1 (as cultivated); 2 (as wild)

Database search information

SciFinder by December 2012. Keywords: *Lepista nuda* or *Clitocybe nuda* or *Tricholoma nudum*. PubMed by December 2012. Keywords: *Lepista* or *Clitocybe* or *Tricholoma*.

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Lepista personata s. auct. non (Fr.) Cooke,

See *Lepista saeva*.

Lepista saeva (Fr.) P.D. Orton
(*L. personata* s. auct. non (Fr.) Cooke, *Tricholoma personatum* s. auct. non (Fr.) P. Kumm.)

Field Blewit (DK: Bleg Hekseringshat, FI: Syysvalmuska, IS: Blásveppur, NO: Lillastilket ridderhatt, SE: Höstmusseron).

Background and risk assessment

Field Blewit (*Lepista saeva*)²⁰ is an edible mushroom.

It is saprotrophic, growing in gardens, parks, roadsides and deciduous woods. It is common in temperate, occasional in hemiboreal/boreal zones of the Nordic countries (Knudsen & Vesterholt, 2012). Occasionally, it is cultivated.

There are no intoxications reported after consumption of Field Blewit, and no natural toxicant constituting a risk for humans has been identified in the mushroom, except that trace amounts of cyanide have been detect-

²⁰ Index Fungorum/Species Fungorum (2012) uses *L. personata* as preferred name but this is wrongly applied for *L. saeva* according to Knudsen & Vesterholt (2012), ArtDatabanken (2011) and Danish Mycological Society (2012).

ed. As the cyanide precursors apparently are effectively destroyed during cooking, the prepared meals will be exempt of cyanide (less than 0.5 mg/kg of prepared meal) and therefore do not pose any hazard in relation to consumption of the cooked mushroom (Stijve & de Meijer, 1999).

Sterner *et al.* (1982) tested an extract of Field Blewit in the Ames test using the *Salmonella typhimurium* strains TA98, TA100 and TA2637. The extract was positive in the *Salmonella* strain TA100. The activity was not enhanced in the presence of microsomal enzymes. Unfortunately, Sterner *et al.* (1982) give no information, whether they had controlled for the potential presence of histidine in the extract. It is well known that false positives may be obtained in the presence of histidine. Also Morales *et al.* (1990) studied mushroom extracts in the Ames test. Extracts of Field Blewit mushrooms were reported to be weakly mutagenic in the *Salmonella* strains TA98 and TA100. The activity was not enhanced by addition of microsomal enzymes. Apparently, Morales *et al.* (1990) did not control for possible histidine in the extracts for the Ames test. Thus, there is no strong indication that extracts of Field Blewit are mutagenic.

Recommendation

No special recommendation.

Listing 2

Database search information

SciFinder by December 2012. Keywords: *Lepista saeva* or *Lepista personata* or *Tricholoma personata*. PubMed by December 2012. Keywords: *Lepista* or *Clitocybe* or *Tricholoma*.

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Lyophyllum connatum (Schumach.) Singer

See *Clitocybe connata*.

Lyophyllum gambosum (Fr.) Singer

See *Calocybe gambosa*.

Macrolepiota olivieri (Barla) Wasser

See *Chlorophyllum olivieri*.

Macrolepiota procera (Scop.) Singer

Parasol (DK: Stor Parasolhat, FI: Ukonsieni, IS: Sóhlífarskermill, NO: Stor parasollsopp, SE: Stolt fjällskivling).

Background and risk assessment

Parasol (*Macrolepiota procera*) is an excellent edible mushroom, but it should be thoroughly cooked, as it is slightly toxic when eaten raw (Roth *et al.*, 1990).

It grows in grassland, dunes, roadsides and open wood. It is common in temperate and occasional in hemiboreal/boreal zones of the Nordic countries, in Norway only in hemiboreal zones (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Parasol, and no natural toxicant constituting a risk for humans has been identified in the mushroom. However, two potentially bioactive protease inhibitors have been isolated from Parasol.

Protease inhibitors: Sabotič *et al.* (2009) isolated several similar cysteine proteinase inhibitors with apparent molecular mass around 19 kDa from fruit bodies of Parasol and called the proteinase inhibitors macrocypins. The amino acid sequence identity between the five macrocypin varieties was 75–86%. Characterization of the macrocypins revealed considerable stability at high temperatures and extremes of pH. Macrocypins were demonstrated to be effective inhibitors of papain and

cysteine cathepsin endopeptidases, and also to inhibit cathepsins B and H, which exhibit both exopeptidase and endopeptidase activities but have low efficiency for others (Sabotič *et al.*, 2009). The same research team also identified another cysteine proteinase inhibitor, clitocypin, in the mushroom (Sabotič *et al.* (2011). The binding of the mycocypin proteinase inhibitors with papain-like cysteine proteases have been studied in crystalized material (Renko *et al.*, 2010). Analysis of mycocypin expression in mature fruiting bodies of Parasol revealed that clitocypin is expressed evenly throughout the fruiting body, while the level of expression of macrocypins varies between tissues, the highest amounts occurring in veil fragments and the ring (Sabotič *et al.*, 2011).

By inhibiting protein degradation, proteinase inhibitors have anti-nutritional effects. The clitocypin retained its activity and dimer composition after boiling in water. It is not known if this is the case also for macrocypin.

However, the potential presence of these protease inhibitors has not been reported to give any discomfort after consumption of Parasol.

Recommendation

No special recommendation.

Listing 1

Database search information

SciFinder by January 2012. Keywords: *Lepiota* or *Macrolepiota*. PubMed by December 2012. Keywords: *Lepiota* or *Macrolepiota*.

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Macrolepiota rachodes (Vittad.) Singer

See *Chlorophyllum rachodes*.

Morchella conica Pers. (*M. elata* Fr.)

Black Morel ("Morel", "Pointed Morel", "Ribbed Morel") (DK: Kegle-Morkel ("Morkel"), FI: Kartiohuhtasieni, IS: Keilumyrkill, NO: Spissmorkel, SE: Toppmurkla).

Background and risk assessment

Black Morel (*Morchella conica*) is, like Morel (*M. esculenta*), a very delicious fungus.

Black Morel and the closely related Morel (*M. esculenta*) are generally referred to as "morels" and evaluated together (see risk assessment of *M. esculenta*). Based on molecular studies these two species can be divided into several more species, which cannot be distinguished morphologically (O'Donnell *et al.*, 2011).

It is not common in the Nordic countries. It can be found in spring. It grows both in deciduous and coniferous forests (Hansen & Knudsen, 2000). Occasionally, it may be found in great amounts on bark chips from coniferous trees. Until now Black Morel has not been very successfully cultivated.

A thorough risk assessment of the consumption of Black Morel is not possible, as the toxicants responsible for the gastrointestinal and/or the neurological effects are unknown. However, based on estimated exposure and human observations on consumption of Black Morel and the closely related Morel it is concluded that Black Morel may give rise to

gastrointestinal effects when eaten raw or insufficiently cooked, with an onset time between less than 1 and up to 6 hours. Intake of higher amounts (more than 100 g per meal) of even well-cooked, fresh morels, or probably also of corresponding amounts of dried morels may in addition give rise to neurologic effects after 6–12 hours latency with ataxia, dizziness and visual disturbances. All symptoms resolve within one day.

Lin *et al.* (2013) have isolated small amounts, up to 35 mg/kg (dry weight, 12 samples) of ergothioneine and of lovastatin, up to 29 mg/kg (dry weight, 12 samples) in mycelia of Black Morel. It is not known, whether these two compounds also may occur in the fruit bodies. For further data on ergothioneine and lovastatin: See *Lepista nuda* and *Pleurotus ostreatus*, respectively.

For a more thorough risk assessment of Black Morel (together with *M. esculenta*), see *M. esculenta*.

Recommendations

Black Morel (*Morchella conica*) and the closely related Morel (*M. esculenta*):

- Should never be eaten raw
- Should be cooked for at least 10 minutes
- Intake of higher amounts (more than 100 g per meal) of cooked, fresh – or corresponding amounts of dried morels – may occasionally, beyond gastrointestinal disturbances, give rise to neurological effects like ataxia, dizziness and visual disturbances and should accordingly be avoided.

Database search information

SciFinder by December 2012. Keywords: *Morchella*.

Listing 1

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Morchella elata Fr.

See *Morchella conica*.

Morchella esculenta (L.) Pers.

Morel (DK: Spiselig Morkel ("Morkel"), FI: Pallohuhtasieni, IS: Steinmyrkill, NO: Rundmorkel, SE: Rund toppmurkla).

Background and hazard identification

Morel is prized as one of the most delicious edible fungi.

Morel (*Morchella esculenta*) and the closely related Black Morel (*M. conica*) are generally referred to as "morels". Based on molecular studies these two species can be divided into several more species, which cannot be distinguished morphologically (O'Donnell *et al.*, 2011).

It is an uncommon wild mushroom, which can be found in spring in rich deciduous forests, parks and gardens, often on chalky soils (Hansen & Knudsen, 2000). Until now morels have not been cultivated very successfully.

Both the fresh and the dried morels are commercially available on the market.

Old publications did report more serious intoxications after eating Morel. This gave rise to the recommendation, that the mushroom should only be eaten after parboiling and removal of the boiling water (Krause, 1918; Aufrecht, 1927). Afterwards, intoxications with gastrointestinal symptoms have been reported occasionally. Newer mushroom books

and other references do not recommend parboiling, but indicate that intake of raw or insufficiently cooked fresh morels may give intoxications (Flammer & Schenk-Jäger, 2009).

Recently, case reports (Pfab *et al.*, 2008) and a 20 years' summary of poisonings with morels collected in French poison control centres (Saviuc *et al.*, 2010) have described a series of well-documented cases with neurological effects after consumption of morels.

Hazard characterisation

Animal, *in vitro*, genotoxicity and metabolism studies

No experimental toxicity data were available for Morel or its constituents.

Human observations

Already in 1889 Roumeguère reported intoxications with morels. Later Krause referred to poisonings after intake of morels, especially if they had not been parboiled before cooking (Krause, 1918; Roumeguère, 1889). In 1927 a serious case of poisoning with canned Morel (*M. esculenta*) was reported, but this intoxication was traced back to the presence of “*Helvella suspecta*” (Aurecht, 1927). Saviuc and colleagues (Saviuc *et al.*, 2010) have reviewed several older reports on morel poisonings (Jaccottet, 1925; Maublanc, 1941; Alder, 1956; Nothnagel, 1962 and Fuste *et al.*, 1979) which all suffer from lack of important data like identification of the consumed mushrooms, cooking conditions and amounts consumed.

A few publications deal with the possible association with alcohol in poisonings with morels (Groves, 1964; Piqueras, 2003 and Beug, 2006; Beug *et al.*, 2006). Whether there is a connection is still an open question.

Recently thorough descriptions of intoxications after consumption of Morel and Black Morel (*M. conica*) have been reported by Pfab and colleagues (Pfab *et al.*, 2008). In six cases people had eaten freshly collected, cooked Morel often in high amounts, up to 250 gram and in one case up to 600 gram and after 6–12 hours latency experienced different neurological effects, typically ataxia and visual disturbances. The effects disappeared within one day after consumption. On request, none of the six persons had eaten any drugs or sedatives, and only two persons had consumed alcohol, one or two glass of beer. In five of the six cases the mushrooms were identified as Morel and in one case as Black Morel, based on macroscopic observations and microscopical identification of the spores. Pfab and co-workers, Department of Toxicology, Klinikum rechts der Isar, Munich also referred to ten similar cases reported from their Poison Control Center

with neurologic effects like dizziness ataxic gait and blurred vision after intake of either Morel or Black Morel (Pfab *et al.*, 2008).

Several morel intoxications with neurological syndrome have been reported by poison control centres in North America (Beug *et al.*, 2006), and in France (Saviuc & Harry, 2008; Saviuc *et al.*, 2010). When detailed information was available, the neurological syndrome was apparently only observed after intake of large amounts of morels, also if they had been well cooked. Most intoxications with morels have gastrointestinal symptoms as their main toxic effect and no neurological symptoms. Poisonings with mainly gastrointestinal symptoms are generally observed after intake of raw or insufficiently cooked morels, even after intake of small amounts (Beug *et al.*, 2006b; Saviuc *et al.*, 2010).

The toxicants responsible for the reported gastrointestinal and neurologic effects are not known, but those responsible for gastrointestinal effects without neurological symptoms are at least partially destroyed or eliminated by cooking (Saviuc & Harry, 2008; Flammer & Schenk-Jäger, 2009). Based on information available, it is anticipated that consumption of dried morels do not give rise to intoxications (Flammer & Schenk-Jäger, 2009), but this is at least not always the case as reported by Beug (2006a) and by Saviuc *et al.* (2010).

In studies on mycelia of Morel, small amounts of ergothioneine have been identified, 19 mg/kg (dry weight, mean of 3 samples) by Lo *et al.* (2012) and up to 35 mg/kg (dry weight, mean of 3 samples) by Lin *et al.* (2013). For further data on ergothioneine: see *Lepista nuda*. Also small amounts of lovastatin, 8–21 mg/kg (dry weight) have been shown in the mycelia (Lin *et al.*, 2013), whereas, Lo *et al.* (2012) identified 1440 mg/kg (dry weight, mean of 3 samples) of lovastatin. For further data on lovastatin: see *Pleurotus ostreatus*. It is not known, whether these bioactive compounds also occur in the fruit bodies of Morel.

Exposure assessment

Morel is a costly mushroom, usually only consumed in small amounts. Generally, intakes are estimated to be less than 50 g fresh or correspondingly smaller amounts of dried mushrooms per meal, but occasionally, it may be consumed in large amounts (about 200–600 g per meal: Pfab *et al.*, 2008) or as “several successive platefuls or meals” (Saviuc *et al.*, 2010).

Risk characterisation

A thorough risk assessment of the consumption of Morel is not possible, as the toxicants responsible for the gastrointestinal and/or the neurological effects are unknown. However, based on estimated exposure and human observations on consumption of Morel (*M. esculenta*) and the closely related Black Morel (*M. conica*), it is concluded that morels may give rise to gastrointestinal effects when eaten raw or insufficiently cooked with an onset time between less than 1 and up to 6 hours. Intake of higher amounts (more than 100 g per meal) of even well-cooked, fresh morels, or probably also of corresponding amounts of dried morels may in addition give rise to neurologic effects after 6–12 hours latency with ataxia, dizziness and visual disturbances. All symptoms resolve within one day.

Recommendations

Morel (*M. esculenta*) and the closely related morel Black Morel (*M. conica*).

- Should never be eaten raw
- Should be cooked for at least 10 minutes
- Intake of higher amounts (more than 100 g per meal) of cooked, fresh – or corresponding amounts of dried morel – may occasionally, beyond gastrointestinal disturbances, give rise to neurological effects like ataxia, dizziness and visual disturbances and should accordingly be avoided.

Database search information

SciFinder by December 2012. Keywords: *Morchella*.

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Paxillus involutus (Batsch.) Fr.

Brown Rollrim (Common Roll-rim, Poison Pax) (DK: Almindelig Netbladhat, FI: Pulkkosieni, IS: Garðlumma Lummusveppur, NO: Pluggsopp, SE: Pluggskivling).

Background and hazard identification

Brown Rollrim (*Paxillus involutus*) has earlier been regarded as an edible mushroom (e.g. Ferdinansen & Winge, 1978; Joly, 1973; Lange, 1970), but is now considered as a poisonous mushroom (Barceloux, 2008; Benjamin, 1995; Breskinsky & Besl, 1985; Flammer, 1983; Roth *et al.*, 1990).

Brown Rollrim grows mycorrhizal with different hosts, especially with Spruce (*Picea*) and Birch (*Betula*), in the north with Dwarf Birch (*Betula nana*). It is very common in temperate and up to boreal zones, and occasional, but locally common, in subarctic to alpine zones of the Nordic countries (Knudsen & Vesterholt, 2012).

Hazard characterisation

Human intoxications

The earliest reports that consumption of Brown Rollrim may give rise to intoxications were published in the mid 1900s and described case reports where the poisoned persons had consumed raw or not sufficiently cooked mushrooms (Grzymala, 1958; Rauschert, 1962; Straus, 1949). In one of these reports, Grzymala (1958) stated that around one third of the intoxications in Poland were due to this mushroom. These intoxications occurred 15 minutes–4 hours after mushroom consumption and gave gastrointestinal symptoms. Subsequently, in the early 1960s, Bschor and colleagues described four intoxications with cooked Brown Rollrim, of which three were fatal. The intoxications occurred shortly after the mushroom consumption and resulted in non-localized abdominal pain, circulatory disturbance, parenchymal damage and fat deposition in blood (Bschor & Mallach, 1963; Bschor *et al.*, 1963). Cochet (1974) and Kubicka & Veselsky (1975) have reviewed a series of intoxications, mainly cases with raw or insufficiently cooked Brown Rollrim. Further insight into the Brown Rollrim intoxications was given by Schmidt *et al.* (1971) who described two cases that developed acute hemolytic anemia after eating the mushroom. In both instances massive hemolysis with subsequent shock and acute renal failure occurred. Specific antibodies were demonstrated in the serum of both patients which, in the presence of Brown Rollrim extracts, caused an immune reaction resulting in agglutination and hemolysis of red blood cells.²¹ A few years later, the same research team presented four new cases that within 1½ hours after consumption of cooked Brown Rollrim experienced urticaria, difficulties to breathe, stomach pain, vomiting and diarrhoea, and hemolysis followed by jaundice. At the time it was already known that extracts of raw Brown Rollrim agglutinates erythrocytes (Krüpe, 1956). When the investigators tested extracts from well-cooked and lyophilized Brown Rollrim on the sera of these individuals, they noted agglutination of erythrocytes and in two cases hemolysis. The antibodies in the patient's sera were identified as IgG. It was hypothesized that a mushroom component forms a hapten-like structure in the serum which subsequently forms a complex with erythrocytes that has the potential to act as antigen. The fatal cases that had been reported earlier were suggested

²¹ In the discussion of the immunological observations, Schmidt *et al.* (1971) mentioned allergic reactions. This reference could have been uncritically transmitted into several publications on Brown Rollrim intoxications.

to have resulted in an immunologic cytotoxic reaction similar to those found in their study.

Okonek (1977) described a further patient suffering from hemolytic syndrome and acute failure to produce urine (anuresis) due to ingestion of cooked Brown Rollrim. The patient recovered completely after treatment of combined hemodialysis and hemoperfusion. Winkelmann *et al.* (1982) reported the death of a previously healthy 49-year old man that fell gravely ill and died 3½ days after repeatedly having eaten Brown Rollrim. The consumption resulted in hemolysis and circulatory shock, leading to acute renal failure, acute respiratory failure and disseminated intravascular coagulation in lungs, kidneys, adrenals, myocardium, liver and spleen. Extensive fat emboli were observed in both lungs. An IgG antibody against the mushroom was demonstrated in the patients serum. Olesen (1991) described a Danish case of Brown Rollrim intoxication. Two hours after having consumed a dish with three fruit bodies the person experienced diffuse abdominal pain, vomiting, diarrhoea, dizziness and powerlessness. Although free hemoglobin could be identified in plasma and urine over the following two days, no organ damage was demonstrated. Both serum creatinine and liver enzymes were normal.

A further case that experienced back pain, general weakness, dizziness, nausea and vomiting following a dinner including sufficiently cooked Brown Rollrim was described by Winkelmann *et al.* (1986). Other family members who ate the same meal had no symptoms. Immunological studies using serum from the patient, Brown Rollrim extracts and various reagents identified IgG specific antibodies, probably occurring in immune complexes. To form such complexes, penetration of intact mushroom antigen through intestinal mucosa into blood or lymph vessels has to be postulated. Such penetration without loss of activity has been demonstrated for other agents. Any further mechanism of the immune reaction must be a speculation but the formation of immune complexes bound to erythrocyte membranes is suggestive (Winkelmann *et al.*, 1982, 1986). Adverse reactions subsequently occur when pre-sensitized persons consume the mushroom. Binding of an immune complex to erythrocytes could then cause intravascular hemolysis by activation of the complement chain. Such a mechanism would explain the short latency between consumption and development of clinical symptoms of shock.

More recently, a Russian report refers to thirty-eight intoxications after consumption of Brown Rollrim and/or another *Paxillus* species, *Paxillus atrotomentosus*. Slight intoxication (acute gastroenteritis) was diagnosed in 17 patients, medium-severe in 13 cases, severe in 6 cases, and

extremely severe in two cases. The mushrooms induced functional disorders in the small intestine, as well as acute renal failure. Two patients died (Musselius *et al.*, 2002).

Paxillus syndrome: In his review of hemolytic mushrooms, Flammer (1983) summarized the immune-hemolytic activity of well-cooked Brown Rollrim in sensitized persons. After many years of being able to consume Brown Rollrim, an intoxication suddenly appears 15 minutes–2 hours after mushroom ingestion and results in symptoms such as collapse, weakness, dizziness, stomach pain, nausea, vomiting, diarrhoea, hemolysis, jaundice, reduced or no urine production, kidney pain and red urine. Erythrocyte hemolysis leads to hemoglobinuria that might require plasma exchange and dialysis. Thus, a prerequisite for the immune-hemolytic anemia, the *Paxillus* syndrome, is the sensitization to a mushroom-antigen that subsequently may lead to hemolysis. The effect is unrelated to that of hemolysing proteins which may be found in raw mushrooms but which are inactivated by cooking. The nature of the mushroom-antigen has not been established. However, it seems as an antigen-antibody complex located on the surface of erythrocyte leads to hemolysis after consumption of Brown Rollrim for a minimum number of times to result in sensitization (Schmidt *et al.*, 1971). Deicher and Stangel (1977), and Winkelmann *et al.*, (1982) have identified the antibody as IgG. However, Lefèvre (1982) were unable to inhibit the IgG-mediated reaction by anti-IgG antibodies.

Experimental studies

Although several studies have been performed with Brown Rollrim or extracts from the mushroom *in vitro* (Habtemariam, 1996), or *in vivo*, (Gilot-Delhalle *et al.*, 1991; Lasota, 1970; Lasota & Fortak, 1965; 1966; 1968; Lasota *et al.*, 1967; Nieminen *et al.*, 1977), these have not been able to explain the mechanism for the *Paxillus* syndrome.

Habtemariam (1996) studied the cytotoxicity of water-, butanol- and ethyl acetate-extracts of Brown Rollrim against cells of murine (L929 and RAW 264.7) and human (HeLa and EAhy926) origin. Whereas water extracts had no influence on cell viability, and the butanol extract had some activity at the highest dose tested (1 mg/ml), the ethyl acetate extract was cytotoxic in a concentration-dependent manner (IC₅₀ values between 125 and 250 µg/ml). Extracts that had been exposed to heat or low pH for four hours had the same effects. Lasota and co-workers published four papers describing the toxicity of dried Brown Rollrim in rats. Subsequently, Nieminen *et al.* (1977) administered to male rats 50 or 250 mg dried homogenized Brown Rollrim in aqueous suspension per kg body weight by gastric intubation for 40 days. In these studies on

non-sensitized rats, no toxic symptoms were observed, and no difference in general behavior, body-weight-gain, relative organ weights, gross appearance, and histopathology of major organs was observed between exposed and control rats.

Causative agents

Possibly, Brown Rollrim contains two types of toxicants. One thermolabile toxicant(s), which give(s) rise to relatively harmless intoxications with gastrointestinal effects after consumption of raw or insufficiently cooked mushrooms and another more thermostabile toxicant(s), which after repeated consumption of the mushroom may give rise to serious intoxications with immune-hemolytic anemia and renal failure, which in some cases have led to shock and death after consumption of even thoroughly cooked mushrooms. However, neither the thermolabile nor thermostabile toxicants have been identified (Antkowiak, 2001; Barceloux, 2008) and only few bioactive constituents are known to occur in Brown Rollrim.

Bioactive constituents

Polyphenols: Two polyphenols, involutin and involutone have been identified in the mushroom (Antkowiak *et al.*, 2003; Edwards *et al.*, 1967; Mikolajczyk & Antkowiak, 2009). Gilot-Delhalle *et al.* (1991) linked the *Paxillus* syndrome to the presence in the mushroom of involutin, the chemical and pharmacological properties of which were demonstrated in the 1960s (Edwards *et al.*, 1967). In order to confirm their hypothesis, these investigators studied the chromosome-breaking activity of extracts of Brown Rollrim in early root tips of the plant *Nigella damascena*. These studies identified chromosome type aberrations both in root tips exposed to non-heated and heated mushroom extracts (Gilot-Dehalle *et al.*, 1991). As both involutin and involutone are heatlabile, they are unlikely to be responsible for the clastogenic effects observed and the *Paxillus* syndrome. The structure of involutin is shown below.

Involutin

Muscarine alkaloids: Trace amounts (totally less than 2 mg/kg fresh weight) of the toxicants L-(+)-muscarine (approximately 4%) and its stereoisomer *epi*-muscarine (96%) have been detected in Brown Rollrim (Stadelmann *et al.*, 1976). However, such low amounts of L-(+)-muscarine and its stereoisomer are *per se* of no toxicological concern in relation to consumption of mushrooms (Bresinsky & Besl, 1985; Stadelmann *et al.*, 1976). The structural formula of L-(+)-muscarine is shown below:

Exposure assessment

There are no intake data available on Brown Rollrim. As neither the thermolabile, nor the thermostable toxicants are known, it is not either possible to indicate the exposure to the toxicants in a meal. It is, however, observed that consumption of even a few finger-thick pieces of the mushroom may give rise to significant gastrointestinal symptoms (Bschor *et al.*, 1963). Repeated intake of even parboiled and sufficiently cooked Brown Rollrim may cause serious, possibly fatal, poisonings in sensitized persons (Bresinsky & Besl, 1985). In these cases also intake of small amounts could lead to severe hemolytic reactions.

Risk characterisation

Consumption of raw or insufficiently cooked Brown Rollrim has given rise to many intoxications with gastrointestinal symptoms. Apparently, repeated consumption of meals even with thoroughly cooked mushrooms may occasionally give rise to more serious intoxications with immune-hemolytic anemia, possibly with subsequent renal damage, shock and even death. Neither heatlabile nor heatstable toxicants have been identified.

Recommendation

Brown Rollrim contains potent, but unknown toxicant(s), which is (are) not efficiently destroyed by cooking, and which after repeated meals may give severe adverse reactions, in some cases deadly.

Listing 4

Database search information

SciFinder by January 2012. Keywords: *Paxillus* and toxicity. *Paxillus* and constituents. PubMed by December 2012. Keywords: *Paxillus involutus*.

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Pholiota mutabilis (Schaeff.) P. Kumm.

See *Kuehneromyces mutabilis*.

Pholiota nameko (T. Itô) S. Ito & S. Imai

(Nameko) “Pholiote” (DK: Nameko-Skælhat, FI: No Finnish name, IS: Namekoskrýfa, NO: (Nameko), SE: Namekotofsskivling).

Background and risk assessment

Pholiota nameko is an edible mushroom.

It is a saprotrophic mushroom and grows wild in deciduous forests in south-eastern Asia, especially Japan (Hongo & Izawa, 2006). It is also widely cultivated, especially in China and Japan.

There are no intoxications reported after consumption of *Pholiota nameko*, and no natural toxicants constituting a risk for humans have been identified in the mushroom. However, two bioactive constituents may occur in *P. nameko*:

Statins: Chen *et al.* (2012) found 186 mg/kg (dry weight, mean of 3 samples) of lovastatin in fruit bodies of *P. nameko*. For further information on statins: see *Pleurotus ostreatus*.

Ergothioneine: Furthermore, Chen *et al.* (2012) found 229 mg/kg (dry weight, mean of 3 samples) of ergothioneine in the fruit bodies of *P. nameko*. For further information on ergothioneine: see *Lepista nuda*.

Recommendation

No special recommendation.

Listing 1**Database search information**

SciFinder by January 2012. Keywords: *Pholiota*. PubMed by December 2012. Keywords: *Pholiota*.

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Pholiota squarrosa (Vahl) P. Kumm.

Shaggy Scalycap (DK: Krumskællet skælhat, FI: Pörhösuomuhelokka, IS: Ígulskrýfa, NO: No Norwegian name, SE: Fjällig tofsskivling).

Background and risk assessment

Shaggy Scalycap (*Pholiota squarrosa*) is no longer considered as an edible mushroom, although it is indicated to be edible in some mushroom textbooks (e.g. Egeland & Myhr, 2003; Gerhardt, 1997; Gulden *et al.*, 1999; Knudsen & Petersen, 2007).

Shaggy Scalycap grows in large clusters at the base of living or recently dead deciduous trees, often on cultivated trees and rather frequently also on Spruce (*Picea*). It is common in temperate and up to middle boreal zones and occasional up to subalpine/subarctic zones of the Nordic countries (Knudsen & Vesterholt, 2012).

Poisonings have been reported after consumption of Shaggy Scalycap (Benjamin, 1995; Beug *et al.*, 2006; Beug, 2009; Danish Veterinary and Food Administration, 2012; Hirohisa, 2000; Shaffer, 1965). Although some people eat this mushroom regularly without discomfort others may have gastrointestinal distress, sometimes severe, with vomiting and diarrhoea, one or more hours after consumption of the mushroom (Aurora, 1986; Benjamin, 1995; Bresinsky & Besl, 1985; Lincoff & Mitchel,

1977; Roth, 1990). Some of the poisonings are associated with alcohol intake along with the mushroom, but others are poisoned without concomitant alcohol consumption (Benjamin, 1995; Bresinsky & Besl, 1985; Haberl *et al.*, 2011; Lincoff & Mitchel, 1977).

No toxicants have been identified in Shaggy Scalycap. However, it contains a lectin with a molecular weight of 4.5 kDa which is stable between pH 2 and 11 and at temperatures ranging from 0 to 100 °C. The lectin binds specifically to α 1-6 fucosylated glycans and agglutinates intact erythrocytes from many animals (Kobayashi *et al.*, 2012). Earlier, Furukawa and colleagues (1995) had shown that a partially purified lectin from the mushroom strongly inhibited human erythrocytes. Some lectins, especially the thermostabile ones, may give rise to gastrointestinal discomfort (Liener, 1983).

Recommendation

Shaggy Scalycap may occasionally give rise to intoxications. The toxicant(s) is(are) unknown. Therefore, the mushroom should not be traded commercially.

Listing 4

Database search information

SciFinder by January 2012. Keywords: *Pholiota*. PubMed by December 2012. Keywords: *Pholiota*.

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Pleurocybella porrigens (Pers.) Singer

Angel's Wings (DK: Kridthat, FI: No Finnish name, IS: No Icelandic name, NO: Krittøstersopp, SE: Öronmussling).

Background and hazard identification

Angel's Wings (*Pleurocybella porrigens*) has generally been considered as an edible mushroom in Europe, North America and Asia (Holmberg & Marklund, 1996; Lincoff, 2000; Læssøe, 1998; Philips, 1991; Philips & Jacobsson, 1996; Sasaki *et al.*, 2006). Especially in Japan, it has been highly appreciated and was widely consumed without any reports on adverse effects (Gejyo *et al.*, 2005; Sasaki *et al.*, 2006; Wakimoto *et al.*, 2011) until an acute outbreak of encephalopathy with several fatalities occurred in northern Japan in 2004 (Gonmori *et al.*, 2011). In Europe, it has been commercialized in the UK for more than ten years. After the reports on encephalopathy with associated fatalities, the sale in UK was suspended (Peebles, 2012). However, the poisonings of Angel's Wings consumers in Japan in 2004 and apparently also a few years later (Takata *et al.*, 2009) have given rise to serious concern about the edibility of this mushroom.

Angel's Wings is a saprotrophic mushroom growing on stumps or logs of coniferous wood. It is occasional in southern parts of Finland, parts of Jutland in Denmark and very common in south-western parts of Sweden

and found in costal parts up to the boreal zone of Norway, but otherwise rare or lacking in the Nordic countries (Knudsen & Vesterholt, 2012).

Chemical investigations have demonstrated several biologically active constituents in Angel's Wings which alone or in combination could be the potential causative agents for the poisonings described. The bioactive constituents include vitamin D analogues (Sasaki *et al.*, 2006), unusual long-chain fatty acids (Amakura *et al.*, 2006a, 2006b; Hasegawa *et al.*, 2007), sialic acids (Takata *et al.*, 2009), lectin (Furukawa *et al.*, 1995; Suzuki *et al.*, 2007), hirsutane-type sesquiterpenoids (Liermann *et al.*, 2011) and β -hydroxyvaline related amino acids (Aoyagi & Sugaharra, 1988; Kawaguchi *et al.*, 2010). In addition, small amounts of cyanogenic compounds occur in the mushroom (corresponding to up to 11 mg hydrogen cyanide per kg fresh weight) has been proposed to be the cause for the encephalopathy (Akiyama & Sasaki, 2007; Akiyama *et al.*, 2006; Gonmori & Yokoyama, 2009), but such low exposures to hydrogen cyanide via consumption of Angel's Wings is unlikely to be of any concern (Stijve & de Meijer, 1999). The most likely causative agent is an unstable aziridine carboxylic acid, isolated in high amounts from Angel's Wings and named pleurocybellaziridine (Wakimoto *et al.*, 2011). It seems probable that this compound may be an unstable common precursor for the β -hydroxyvaline related amino acids isolated from Angel's Wings by Kawaguchi *et al.* (2010). *In vitro* studies exposing rat oligodendrocytes to pleurocybellaziridine showed that that the compound significantly reduced the viability of the cultivated cells, whereas the β -hydroxyvaline carboxylic acids formed from pleurocybellaziridine were significantly less cytotoxic (Wakimoto *et al.*, 2011).

The chemical structures of pleurocybellaziridine and one of the β -hydroxyvaline related amino acids are shown below.

Hazard characterisation

Human observations

At least 55 cases with encephalopathy including at least 19 deaths, most due to respiratory failure, have been reported after consumption of Angel's Wings in Japan in 2004 (Gejyo *et al.*, Gonmori & Yokoyama, 2009; Kawaguchi *et al.*, 2010; Kato *et al.*, 2004; Obara *et al.*, 2005; Saviuc & Danel, 2006; Takata *et al.*, 2009) and apparently also in 2007 (Takata *et al.*, 2009). Nearly all of the poisoned persons already experienced chronic renal failure and many were undergoing hemodialysis treatment.

The onset of symptoms varied from one to 31 days (mean 9 days) (Gejyo *et al.*, 2009) or 2–3 weeks (Kato *et al.*, 2004; Kurakawa *et al.*, 2005) after consumption of the mushroom. The symptoms were not gastrointestinal or hepatic symptoms commonly seen in mushroom poisonings. Instead a number of neurological symptoms were reported, such as disturbed consciousness, weakness of extremities, convulsions, myoclonus, dysarthria, ataxia and paresis/paralysis (Gejyo *et al.*, 2005; Kato *et al.*, 2004; Kurokuwa *et al.*, 2005). CT and MR brain scans were normal the first days after onset of symptoms, but later different brain lesions were observed. Approximately 30% of the patients who had presented with encephalopathy after ingestion of Angel's Wings died, in most cases after respiratory failure (Gejyo *et al.*, 2005; Kato *et al.*, 2004; Obara *et al.*, 2008; Saviuc & Danel, 2006).

Based on the data available, it has not been possible to establish a dose-response relationship between the amount of mushrooms consumed and the severities of the encephalopathy. However, the degree of renal failure is apparently essential for the prognosis of this type of encephalopathy (Kato *et al.*, 2004; Saviuc & Danel, 2006).

Histological examination of the brain of patients affected by encephalopathy show symptoms of demyelination (Wakimoto *et al.*, 2011). This indicates that toxic constituent(s) in the mushroom has (have) damaged oligodendrocytes in the brain. Therefore, Wakimoto and colleagues (2011) studied the toxicity of the suspected causative agent pleurocybellaziridine against rat CG4-oligodendrocytes cultured *in vitro*. The cell viability was significantly reduced at concentrations of 10 mg/ml pleurocybellaziridine. At a concentration of 30 mg/ml most of the cells died. Pleurocybellaziridine seems to be a precursor for at least five unusual amino acids which all contain a 2-hydroxyvaline moiety. These unusual amino acids have been shown to be cytotoxic against mouse cerebrum glial cells (Kawaguchi *et al.*, 2010), but they exhibited much less toxicity than pleurocybellaziridine against rat CG4-16 oligodendrocytes. It is therefore indicated that pleurocybellaziridine is the causative constituent or at least contributes to the observed encephalopathy after consumption of Angel's Wings.

Animal studies

In an *in vivo* study with two strains of mouse, oral administration of 5 g/kg body weight/day of an aqueous extract of Angel's Wings (extracted by 90 °C for 30 minutes) showed no toxicity. However, an intraperitoneal injection of 1 g/kg bodyweight of the same extract caused anemia, shock and death, whereas an intraperitoneal injection of an aqueous extract that had been prepared at 100 °C for 30 minutes had no significant toxic effects. These data indicate that the toxicant(s) in the mushroom might be thermolabile (Takano *et al.*, 2005).

Exposure assessment

Mushroom consumption: Although Angel's Wings is common in Finland, Norway and Sweden (rare in Denmark and not found in Iceland) and indicated to be edible in some Nordic mushroom textbooks (Holmberg & Marklund, 1996; Læssøe, 1995; Philips & Jacobsson, 1996), it has not been included in earlier Nordic guidance lists on mushrooms for consumption, and it has not been marketed in the Nordic countries. Therefore, it is estimated that the Nordic consumption of this mushroom is very modest, if occurring at all. However, in other countries, especially in Japan, it is a popular mushroom considered to be edible. In the autumn of 2004, when most of the intoxications due to Angel's Wings consumption in Japan took place, the mushroom was extraordinary abundant due to special weather conditions and it is possible that it was eaten in high amounts. However, there are no specific data available on consumption.

Content of anticipated causative agent: As mentioned above, Wakimoto and colleagues have recently isolated an aziridine carboxylic acid ester from an esterified extract of Angel's Wings in amounts corresponding to 2.3 g pleurocybellaziridine per kg fresh mushroom. It was necessary to esterify the mushroom sample as the free pleurocybellaziridine otherwise will be destroyed during the isolation process. It was also mentioned above, that *in vitro* studies indicate that this compound is the causative agent for the encephalopathy after consumption of Angel's Wings in Japan.

It is not possible to estimate the content of pleurocybellaziridine in the Angel's Wings samples consumed and giving rise to the intoxications in 2004. The content has been determined only once, in mushrooms, apparently collected several years after the outbreak of poisonings in 2004 (Wakimoto *et al.*, 2011). It has been proposed that there are different varieties of Angel's Wings in Japan, and it is conceivable that these contain different amounts of the causative agent (Matsumoto *et al.*,

2005). As the toxicant seems to be destroyed by heat (Takano *et al.*, 2005; Wakimoto *et al.*, 2011), the cooking of Angel's Wings, although traditionally for a very short period (Wakimoto *et al.*, 2005), could lead to a reduction in the content of the toxic compound.

Risk characterisation

Based on human observations it is highly likely that consumption of Angel's Wings has given rise to at least 55 cases of encephalopathy. At least 19 of the affected persons died. Most of the patients had a history of chronic renal failure and many underwent hemodialysis.

The anticipated causative agent is an unstable aziridine carboxylic acid, pleurocybellaziridine, which may occur at high levels in the mushroom. However, neither the amounts of mushrooms consumed by the poisoned persons, nor the level of pleurocybellaziridine in the consumed mushrooms are known.

Uncertainties: As Angel's Wings has been consumed for many years in Europe, Asia and North America without any intoxications having been reported before the epidemic outbreak of encephalopathy in Japan in 2004 (and apparently also some in 2007), the Asian cases have raised several questions and resulted in uncertainties whether it is appropriate to consume the mushroom (Beug, 2011; Gejyo *et al.*, 2005; Saviuc & Danel, 2006):

- Was the content of toxicants in the mushroom extraordinary high in 2004 due to the extraordinary hot and humid weather conditions this year?
- Was the consumption exceptionally high in 2004, as the Angel's Wings could be collected in large quantities this year?
- Is pleurocybellaziridine the causative agent (the anticipation is mainly based on cell culture studies and data from very limited studies in mice)?
- Do all collections of Angel's Wings contain the agent (pleurocybellaziridine) that caused the outbreak of intoxications in 2004 or are there various forms of the mushroom?
- What is the mechanism of the acute encephalopathy after consumption of Angel's Wings?

Recommendation

Consumption of Angel's Wings has given rise to several serious and sometimes fatal intoxications, most likely due to its content of pleurocybellaziridine. With the present knowledge, Angel's Wings should not be marketed as an edible mushroom.

Listing 4

Database search information

PubMed by December 2012. Keywords: *Pleurocybella*. SciFinder 2011–2012. Keywords: *Pleurocybella*.

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Pleurotus citrinopileatus Singer

Golden Oyster (DK: Gul Østershat, FI: Sitruunavinokas, IS: Gulvængu, NO: Sitronøsterssopp, SE: Citronmussling).

Background and risk assessment

Golden Oyster (*Pleurotus citrinopileatus*) is an edible mushroom, which is commonly cultivated and consumed.

It is a saprotrophic mushroom, growing wild in deciduous forests in Japan and far-east Russia (Hongo & Izawa, 2006). It is mainly the cultivated form, which is commercially available.

There are no intoxications reported after consumption of Golden Oyster, and no natural toxicants constituting a risk for humans have been identified in the mushroom. However, Golden Oyster contains different bioactive constituents:

Bioactive constituents

Statins: Chen *et al.* (2012) have found 120 mg/kg (dry weight) of lovastatin in fruit bodies of Golden Oyster. Lovastatin has also been identified in an amount of 930 mg per kg (dry weight) in mycelia (Lo *et al.*, 2012), whereas Lin *et al.* (2013) could not detect any lovastatin in mycelia from this mushroom. When statins like lovastatin are used as a cholesterol-lowering drug, it has in rare cases given rise to adverse effects with my-

opathy, characterised by muscular weakness and/or pain, and in very rare cases rhabdomyolysis.²² These serious adverse effects have not been reported after consumption of Golden Oyster. For further data on lovastatin, see *P. ostreatus*.

Hydrogen cyanide: Golden Oyster has been demonstrated to produce low amounts of hydrogen cyanide. However, during cooking the cyanogenic compound is completely destroyed and accordingly does not pose any health hazards to the consumers (Stijve & de Meijer, 1999).

Lectins: Li *et al.* (2008) have isolated a dimeric lectin with a molecular weight of 32.4 kDa from the mushroom. The lectin has high hemagglutinating activity and is stable up to 60 °C. Whether this lectin could give rise to discomfort in people eating Golden Oyster raw or insufficiently cooked is not reported. It is well-known that some other lectins in certain foods may cause gastrointestinal effects (Liener, 1983).

Ergothioneine: It has been shown that Golden Oyster contains 2,850 mg/kg (dry weight) of ergothioneine in the fruit bodies (Chen *et al.*, 2012) and 1,000 mg/kg (dry weight) in the mycelia (Lin *et al.*, 2013) or 26 mg/kg (dry weight) in the mycelia (Lo *et al.*, 2012). For further information on ergothioneine, see *Lepista nuda*.

Experimental studies: Fractions of aqueous extracts from fruit bodies of Golden Oyster significantly lowered serum triglycerides and total cholesterol when fed as a supplement to high-fat diets to hyperlipidemic rats. Furthermore, high-density lipoprotein levels were significantly higher than in the control groups (Hu *et al.*, 2006). There was no information on possible content of statins in the diets used.

Recommendation

No special recommendation.

Listing 1

Database search information

PubMed by 1 December 2012. Keywords: *Pleurotus citrinopileatus*.

²² Rhabdomyolysis is the breakdown of muscle fibers that leads to the release of muscle fiber contents (myoglobin) into the blood stream. Myoglobin is harmful to the kidney and often causes kidney damage (MedlinePlus Medical Encyclopedia, 2012).

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Pleurotus djamor (Rumph. ex. Fr.) Boedijn
(*P. salmoneostramineus* Lj. N. Vassiljeva)

Tropical Oyster (DK: Rød Østershat, FI: No Finnish name, IS: Rodavængur, NO: Flamingøsterssopp, SE: (Rosa ostronmussling)).

Background and risk assessment

Tropical Oyster (*Pleurotus djamor*) is an edible mushroom, which is commonly cultivated and consumed.

It is a saprotrophic mushroom, growing wild, on wood, in China, Sri Lanka and Japan (Anonymous, 1986; Pegler, 1986).

There are no intoxications reported after consumption of Tropical Oyster, and no natural toxicants constituting a risk for humans have been identified in the mushroom. There are only few studies on bioactive constituents in Tropical Oyster:

Bioactive constituents

Ergothioneine: Chen *et al.* (2012) detected 1,250 mg/kg of ergothioneine in the fruit bodies (dry weight) and Lo *et al.* (2012) 220 mg/kg in the mycelia (dry weight). For further information on ergothioneine, see *Lepista nuda*.

Statins: Neither Chen *et al.* (2012) analysing fruit bodies, nor Lo *et al.* (2012) and Chegwin-Angarita *et al.* (2013) analysing cultured mycelia

could detect any lovastatin in Tropical Oyster. Most other *Pleurotus* species studied contain lovastatin (Lin *et al.*, 2013).

Experimental studies: In a study on hypolipidemic (and anti-atherogenic) effects of Tropical Oyster, hypercholesterolemic rats were fed 5% dried, powdered fruit bodies of the mushroom. Plasma total cholesterol, triglycerides and cholesterol in low-density-lipoprotein (LDL) were significantly reduced when compared with rats fed a control diet. The authors did not analyse for lovastatin in the mushrooms used for the rat study (Yoon *et al.*, 2012).

Recommendation

No special recommendation.

Listing 1

Database search information

PubMed by 1 December 2012. Keywords: *Pleurotus djamor*, *Pleurotus salmoneostramineus*.

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Pleurotus eryngii (DC.) Quél. (*P. eryngii* var. *ferulae* (Lanzi) Sacc.)

King Oyster (French Horn Mushroom, King Trumpet Mushroom) (DK: Kejser-Østershat (Mandstro-Østershat, "Kejserhat"), FI: Kuningas-osterivinokas (Kuningasvinokas), IS: Istruvængur, NO: Kongeøsterssopp, SE: ("Kungsmussling")).

Background and risk assessment

King Oyster (Pleurotus eryngii) is an edible mushroom, which is commonly cultivated and consumed.

It is a saprotrophic mushroom growing on dead (apparently also on living/dying) tall umbelliferous herbs, e.g. on species of the genera *Eryngium* and *Ferula* in open steppe-like areas. The mushroom grows wild in the Mediterranean area and eastwards into China and Central Asia (Hongo & Izawa, 2006).

King Oyster is a complex of varieties or possibly species, including *P. eryngii* var. *eryngii* and *P. eryngii* var. *ferulae*, but classification is still under discussion (Stajic *et al.*, 2009).

There are no intoxications reported after consumption of King Oyster, and no natural toxicants constituting a risk for humans have been identified in the mushroom. However, different bioactive constituents have been identified in King Oyster.

Bioactive constituents

Statins: Between 120 and 150 mg lovastatin per kg (dry weight, 9 samples) have been detected in dried fruit bodies of King Oyster by Chen *et al.* (2012), whereas these investigators found lower levels in the mycelium 45 mg per kg (dry weight, 3 samples) in mycelia. Lo *et al.* (2012) and Lin *et al.* (2013) have confirmed the presence of lovastatin in mycelia from King Oyster, the levels being 67 and 42 mg per kg (dry weight, 3 samples) in mycelium, respectively. For further information on statins: see *Pleurotus ostreatus*.

Alam *et al.* (2011) studied the antihyperlipidemic effects of King Oyster in hypercholesterolemic rats and noted that feeding a diet containing 5% dried powdered fruit bodies of the mushroom, reduced significantly plasma total cholesterol, triglycerides, low-density-lipoprotein (LDL) and other lipid parameters. It was hypothesized whether these effects could partly be explained by the content of lovastatin.

The structural formula of lovastatin is shown below.

Lovastatin

Ergothioneine: In one study Chen *et al.* (2012) identified 460–840 mg/kg (dry weight, 9 samples) of ergothioneine in the fruit bodies and 1,500 mg/kg (dry weight, 3 samples) in mycelia of King Oyster. Lo *et al.* (2012) and Lin *et al.* (2013) found somewhat less, 250 and 240 mg/kg (dry weight) in the mycelia, respectively.

Cytolysins: A monomeric hemolysin, designated eryngeolysin, with a molecular weight of 17 kDa has been isolated from King Oyster fruit bodies. It has structural similarities with the hemolysin, ostreolysin, from *P. ostreatus*. It is stable in the pH range 4.0–12.0, but only stable up to 30 °C (Ngai & Ng, 2006). More recently, Shibata *et al.* (2010) isolated and characterized a thermostable two-component hemolysin constituted by erylysin A (molecular weight 15 kDa) and erylysin B (molecular weight 52 kDa). Erylysin A was a homodimer, whereas erylysin B was a

heterodimer of the B₁ (molecular weight 15 kDa) and B₂, (molecular weight 37 kDa) components. Erylysin (A and B) is similar to the hemolysin, pleurotolysin (A and B) isolated and characterized by Tomita *et al.* (2004) in *P. ostreatus*. Both hemolysins require coexistence of the A and B units to exhibit strong activity.

There are no intoxications reported after consumption of thoroughly cooked King Oyster. This indicates that any potential effects of the cytoly-sins are eliminated during heating and/or digestion.

α,β-Unsaturated lactones: Three *α,β*-unsaturated lactones have been isolated and identified from a solid culture of King Oyster, eryngiolide A, 1,2-dihydroxymintlactone and 5-hydroxy-3,4,5-trimethylfuran-2(5H)-one. An *α,β*-unsaturated lactone moiety is regarded as a significant structural characteristic for many naturally occurring bioactive compounds. EFSA (2007) considers the *α,β*-unsaturated ketone structure as a structural alert for genotoxicity. The three lactones were tested for cytotoxic activity against the two human cancer cell lines HeLa and HepG2. Only eryngiolide A showed toxicity against the cell lines (Wang *et al.*, 2012). Eryngiolide A is a diterpenoid with a cyclodecane skeleton, substituted with several functional groups and fused with two lactone groups. The structural formula for eryngiolide A is shown below.

Eryngiolide A

A further *α,β*-unsaturated lactone (4H-1,3-dioxine-2,4-dione), designated pleurone, has been isolated from fruit bodies of *P. eryngii* var. *ferulae* (King Oyster). Pleurone was shown *in vitro* to be a moderate human neutrophil elastase inhibitor (Lee *et al.*, 2011). As none of these four *α,β*-unsaturated lactones have been studied *in vivo*, it is difficult to estimate if these compounds could be of health concern for humans.

Cyanogenic compounds: Shindo and colleagues examined the content of cyanide in different Basidiomycetes. The content of cyanide in King Oyster was in average 14 mg per kg of fresh mushroom (7 samples,

range 1–26 mg/kg). The chemical form was probably as free cyanide, because there was no significant difference in quantities of cyanide before and after incubation with glucosidases (Shindo *et al.*, 1999). Subsequent studies by Chou *et al.* (2006) in 52 samples of fresh King Oyster mushrooms collected from the local market in Taiwan or mushroom farms, showed that most (67%) products contained hydrogen cyanide concentrations below 1 mg/kg, 17% of the samples levels between 1.0 and 2.0 mg/kg, and only 15% of samples more than 2.0 mg/kg. When the mushroom was sliced and cooked in water at 95 °C for 6 minutes, 89% of the original hydrogen cyanide was lost (Chou *et al.*, 2006). Thus, it is estimated that the exposure to hydrogen cyanide after consumption of King Oyster, especially after cooking, would be so low that it is of no health concern.

Mutagens: Morales *et al.* (1990a, 1990b) studied mushroom extracts in the Ames test. Extracts of freshly harvested and frozen King Oyster were reported to be weakly mutagenic in the *Salmonella* strains TA98 and TA100. Extracts of canned King Oyster were negative in the Ames test but weakly mutagenic, however only after addition of microsomal enzymes, in the Chinese Hamster Ovary (CHO/HPRT) cell mutation assay. Apparently, Morales *et al.* (1990a, 1990b) did not control for possible histidine in the extracts for the Ames test. Thus, there is no strong indication that extracts of King Oyster are mutagenic.

Recommendation

No special recommendation.

Listing 1

Database search information

PubMed by 1 December 2012. Keywords: *Pleurotus eryngii*, *Pleurotus ferulae*.

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P. eryngii var. *ferulae* (Lanzi) Sacc.

See *P. eryngii*

Pleurotus ostreatus (Jacq.) P. Kumm.

Oyster Mushroom (DK: Almindelig Østershat, FI: Osterivinokas, IS: Osturvængur, NO: Blågrå østerssopp, SE: Ostronmussling (Ostronskivling)).

Background and risk assessment

Oyster Mushroom (*Pleurotus ostreatus*) is an edible mushroom. It is one of the most commonly cultivated and consumed mushrooms. In 1997, the year from which the latest world production figures were available, it was cultivated in 875,000 tons (Chang & Miles, 2004).

It is a saprotrophic or weakly parasitic mushroom, which grows imbricate in groups on trunks of living and dead deciduous woods, rarely on conifers. It is common in temperate, up to southern boreal zones and is occasional in middle boreal up to subarctic/subalpine zones of the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of cooked Oyster Mushroom. However, there are some indications that consumption of large amounts of raw Oyster Mushroom may give adverse effects (Al-Deen *et al.*, 1987; Juntos *et al.*, 2009). Several bioactive constituents have been identified in the mushroom:

Bioactive constituents

Cyanogenic compounds: Traces of hydrogen cyanide (less than 1 mg/kg) have been identified in fresh Oyster Mushroom (Shindo *et al.*, 1999). Such low levels are of no health concern in relation to consumption of the mushroom.

Ergothioneine: Ergothioneine has been demonstrated in Oyster Mushroom fruit bodies at levels in the range 940–2200 mg/kg (dry weight) corresponding to approximately 90–220 mg/kg in the raw mushroom (Chen *et al.*, 2012; Dubost *et al.*, 2006; Lee *et al.*, 2009) which agree with the finding 119 mg/kg in raw fruit bodies (Ey *et al.*, 2007). Much less could be found in the dried mycelia, 78–120 mg/kg (Lo *et al.*, 2012) and 220 mg/kg (Lin *et al.*, 2013), respectively. For further information on ergothioneine, see *Lepista nuda*.

Cytolysins: As mentioned above, there are some indications that intake of large quantities of raw or insufficiently cooked Oyster Mushroom may give rise to intoxications (Al-Deen *et al.*, 1987; Juntres *et al.*, 2009). After oral or intraperitoneal administration of an Oyster Mushroom extract, the LD₅₀ in both cases exceeded 3,000 mg/kg body weight. The extract was prepared at room temperature in order not to denature suspected proteinaceous toxins. Oral or intraperitoneal administration of up to 1,000 mg mushroom extract/kg body weight/day to mice for 30 days resulted in hemorrhages in the intestine and in other tissues. The main histological findings were pathological hepatic changes, but apparently not in a dose-dependent manner (Al-Deen *et al.*, 1987).

Berne *et al.* (2002) have isolated and characterized a thermolabile protein, ostreolysin, having a molecular weight of 15 kDa. It is a cytolysin which lyses bovine, sheep and human erythrocytes *in vitro* and probably also have similar effects *in vivo* as indicated by increased serum potassium (Zuzek *et al.*, 2006). After parenteral application of ostreolysin to rats endothelial injury with oedema in the heart and lungs, as well as myocardial hemorrhages could be demonstrated (Juntres *et al.*, 2009). In 2004, Tomita and colleagues isolated and characterized a two-component, pore-forming cytolysin, pleurotolysin, which consists of two, non-associated components, pleurolysin A (molecular weight 17 kDa) and pleurolysin B (molecular weight 52 kDa). Cooperatively, these two components cause leakage of potassium ions from human erythrocytes and swelling, leading to hemolysis (Tomita *et al.*, 2004). A hemolysin (pleurotolysin) isolated from Oyster Mushroom by Bernheimer & Avigad (1979) is anticipated to be a proteolytic product of the pleurotolysin characterized by Tomita *et al.* (2004). There are no intoxications reported after consumption of thoroughly cooked Oyster Mushroom. This indi-

cates, that any potential effects of the cytolsins are eliminated during heating and/or digestion.

Indole constituents: Oyster Mushroom contains a series of simple biologically active indole compounds (5-hydroxytryptophan, serotonin and tryptamine) (Muszynska *et al.*, 2011, 2012). It was demonstrated that boiling of Oyster Mushroom fruit bodies significantly changed the content of these indoles. 5-Hydroxytryptophan and serotonin were completely decomposed and tryptophan and 5-methyltryptophan had been formed in amounts of 81 mg and 38 mg/kg (dry weight) corresponding to approximately 8 and 4 mg/kg (fresh weight), respectively. Some of these constituents are tissue hormones and neurotransmitters and high intakes of tryptophan can damage the nervous system (Stone *et al.*, 2003).

Mutagens: Morales *et al.* (1990a, 1990b) studied mushroom extracts for mutagenic activity in the Ames test, which detects revertants from histidine dependence to histidine auxotrophy. Extracts of freshly harvested and frozen Oyster Mushroom were reported to be mutagenic in *Salmonella* test strains TA98 and TA100. Extracts of canned Oyster Mushroom were negative in the Ames assay, but weakly positive in the Chinese Hamster Ovary (CHO/HPRT) cell mutation assay. The authors indicate, it is possible that the weak positive response obtained in the test assay may be an artefact. Apparently, Morales *et al.* (1990a, 1990b) did not control for possible presence of histidine in the extracts tested in the Ames test. Therefore, the positive response could be due to histidine in the test solution allowing the detector strain of *Salmonella* bacteria to grow and form colonies. Grüter *et al.*, (1991), using a histidine-independent bacterial test system, identified no mutagenic activity in extracts of Oyster Mushroom. It is concluded that extracts of Oyster Mushroom are probably not mutagenic.

Statins: Oyster Mushroom may contain relatively high amounts of the statin lovastatin. Natural statins are hexahydronaphthalene derivatives, substituted with a β -hydroxylactone. Statins are widely used as hypolipidemic drugs for lowering cholesterol. Lovastatin and simvastatin are examples of statins and are used in daily doses in the range 10 to 80 mg. In certain cases statins may give rise to myopathy, characterised by muscular weakness and/or pain and in very rare cases rhabdomyolysis²³ (e.g., FDA, 2011a, 2011b, 2013; Floyd *et al.*, 2012; Mastaglia &

²³ Rhabdomyolysis is the breakdown of muscle fibers that leads to the release of muscle fiber contents (myoglobin) into the blood stream. Myoglobin is harmful to the kidney and often causes kidney damage (MedlinePlus Medical Encyclopedia, 2012).

Needham, 2012). These serious adverse effects have not been reported after consumption of Oyster Mushroom.

Lovastatin was originally isolated from *Penicillium citrinum* by Endo *et al.* (1976). Later, it has also been identified in other microorganisms and in 1993–95 Gunde-Cimerman *et al.*, (1993a; 1993b) and Gunde-Cimerman & Cimerman (1995) reported on the isolation of lovastatin from different *Pleurotus* species, including Oyster Mushroom. They detected 2,000 to 3,000 mg/kg (dry weight) in the pileus without lamellae and between 2,500 and 6,000 mg/kg (dry weight) in the lamellae. Levels were lower in the stipe; up to 1,000 mg/kg (dry weight) in the lowest part (Gunde-Cimerman *et al.*, 1995). These figures indicate that the fresh fruit bodies would contain approximately 200–300 mg lovastatin/kg. In another study, Alarcon *et al.* (2003) detected 0.40–2.1% of the dry matter of Oyster Mushrooms cultivated on straw to be lovastatin. The corresponding amounts of lovastatin in Oyster mushrooms collected from the forest was 0.7–2.8% of dry matter. These contents correspond to approximately 400–2800 mg lovastatin/kg fresh mushroom. Lower lovastatin levels were found in dried samples of cultivated Oyster Mushroom by Chen *et al.* (2012), 165 mg/kg mushroom in a Korean strain, 607 mg/kg mushroom in a Japanese strain, and 216 mg in a Taiwanese strain, corresponding to approximately 17, 61 and 22 mg/kg in the fresh mushroom, respectively. Lo *et al.* (2012) examined the mycelia of cultivated Korean and Taiwanese strains of Oyster Mushroom, and showed these to contain 60 and 147 mg lovastatin/kg dry weight, respectively, and Lin *et al.* (2013) mycelia from an Israeli strain to contain 222 mg/kg (dry weight). However, Chegwin-Angarita *et al.* (2013) were unable to detect any statin in another strain of Oyster Mushroom. They could neither find it in the cultivated mycelia nor in broth. In a study on lipid lowering effects of Oyster Mushroom in humans, Schneider *et al.* (2011) were unable to detect lovastatin in lyophilized fruit bodies of Oyster Mushroom used in the study diet. Altogether, it seems that some cultivars of Oyster mushroom do not produce lovastatin, whereas others produce quite high amounts.

Food processing may destroy biologically active constituents in mushrooms, e.g., many lectins and other bioactive proteins. Ulziiargal *et al.* (2013) did not find any or very little lovastatin (0.05 mg/kg) in bread added 0.7–0.9 mg/lovastatin per kg bread before baking. However, it is difficult, based on this study, to predict to what extent the much higher amounts of lovastatin, e.g., 400–2,800 mg/kg in fresh Oyster Mushroom (Gunde-Cimerman & Cimerman, 1995; Alarcon *et al.*, 2003) will be chemically changed or extracted during cooking of the mushroom. Furthermore,

the analytical method used by Ulziijargal *et al.* (2013) does not take into account, that the lactone lovastatin during baking processes may be hydrolysed to the corresponding hydroxycarboxylic acid, the active cholesterol lowering form of the statin. Lovastatin is stable in aqueous neutral or acidic solutions at room temperature (Piecha *et al.*, 2010), but 25% is hydrolysed after one hour's heating at 100 °C (Yang *et al.*, 2007). In a thermal and kinetic study on statins, lovastatin was shown to be stable up to 190 °C under solvent-free conditions (Souza *et al.*, 2007).

Altogether, it is likely that a considerable proportion of lovastatin in the mushroom is stable during cooking or possibly to some extent hydrolysed to the hydroxycarboxylic acid, which is the biologically active form of lovastatin.

Human observations: Schneider *et al.* (2011) studied the lipid lowering effects of Oyster Mushroom in humans. Twenty adults (20–34 years) were randomized to consume tomato soup with or without 30 g dried Oyster Mushroom (corresponding to 300 g fresh mushrooms) on a daily basis for 21 days. In the group receiving Oyster Mushroom serum triglycerides, oxidized low density lipoprotein and total cholesterol values were significantly lower, than in the control group. It is remarkable, that the Oyster Mushroom cultivar used for the study did not contain any detectable level of lovastatin. The described effects on the serum lipid parameters must therefore be attributed to other constituents in the Oyster Mushroom diet.

Experimental studies: Several experimental studies in rats and rabbits (Alam *et al.*, 2011; Anandhi *et al.*, 2013; Bobek & Galbavy, 1999; Bobek *et al.*, 1998; Hossain *et al.*, 2003)) indicate that supplying Oyster Mushrooms in the diet may alter the serum lipid profile, including lowering of serum total cholesterol, very-low-density-lipoprotein (VLDL) cholesterol and low-density-lipoprotein (LDL) cholesterol. This effect can, at least partly, be explained by the usually high content of lovastatin in Oyster Mushroom.

Nieminen and co-workers (2009) have published a study on the potential myo- and hepatotoxic effects of different cultivated mushrooms in mice. The animals (six females per group) were for 5 days supplied large quantities of dried and powdered cultivated mushrooms in the feed. One of the cultivated mushrooms, Oyster Mushroom, was given as 1.8%, 3.6% or 5.4% of the feed. The feeding resulted in mice being exposed to 18, 28 and 38 g, respectively, which is equivalent to humans ingesting 1.2, 2.0 or 2.7 kg Oyster Mushroom per person (70 kg) per day or when corrected for body surface constant 102, 164 or 224 g per person (70 kg) per day of fresh Oyster Mushroom, respectively. All mice were healthy at the end of the study and no pathological changes were observed in the histological studies of organs. Of the many clinical pa-

rameters studied, plasma total cholesterol was reduced, reaching statistical significance at the highest dose. The cholesterol lowering effect was possibly due to content of the statin, lovastatin (Nieminen *et al.*, 2009).

The structural formula of lovastatin is shown below.

Lovastatin

Recommendation

No special recommendation.

Listing 1

Database search information

OECD database search by June 2010 (OECD, 2012). PubMed: 2010–2012.

Keywords: *Pleurotus ostreatus*.

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Pleurotus salmoneostramineus Lj. N. Vassiljeva

See *Pleurotus djamor*.

Rozites caperatus (Pers.) P. Karst.

See *Cortinarius caperatus*.

Russula Pers.

Brittlepill (DK: Skørhat, FI: Hapero, IS: Hnefla, NO: Kremle, SE: Kremla).

The genus Brittlepill (*Russula*) comprises 152 species in the Nordic countries. They form mycorrhiza with the coniferous trees Spruce (*Picea*) and Pine (*Pinus*) and with many deciduous trees, especially with Beech (*Fagus*), Birch (*Betula*) and Oak (*Quercus*). The Brittlepill (*Russula*) and Milkcap (*Lactarius*) genera are related and belong to the same family *Russulaceae*. The fruit bodies of the Brittlepill genus are characterized by their crumbling texture, but contrary to the Milkcap species, they do not exude milky juice (latex) after injury. The taste of the different species varies from mild to very pungent/acrid, rarely bitter or oily (Knudsen & Vesterholt, 2012). Furthermore, the Brittlepill genus contains pteridine derivatives (russupteridines) as some of the major colouring matters. These nitrogen containing pigments have not been found in the Milkcap genus (Eugster, 1973; Gluchoff & Lebreton, 1970; Iten *et al.*, 1973; 1984).

The structural formulae of one of the yellow pteridines, riboflavin, and a part of the dimeric structure of a red pteridine, a lumazine derivative, are shown below.

Generally, mild tasting raw Brittlegill species are considered as edible. However, there are some exceptions. Thus, the mild tasting *Russula subnigricans* Hongo, which is widely distributed in Asia, and also found in North America, but not in Europe, has given rise to many severe intoxications in Japan and Taiwan. It looks very much like Blackening Brittlegill (*R. nigricans*), but *R. subnigricans* is only reddening not blackening as Blackening Brittlegill, when bruised (Gonmori *et al.*, 2011). The intoxications have included gastrointestinal symptoms within 2 hours and in several cases also rhabdomyolysis²⁴ within 6 hours after ingestion of the mushroom (Lee *et al.*, 2001, Matsuura *et al.*, 2009). Eight of 20 rhabdomyolysis cases described in Japan between 1955 and 2007 after consumption of *R. subnigricans* were fatal (Gonmori *et al.*, 2011). The rhabdomyolysis occurring after *R. subnigricans* consumption was apparently induced by a different mechanism than rhabdomyolysis observed after Yellow Knight (*Tricholoma equestre*) consumption (Saviuc & Danel, 2006). Based on studies in mice, the causative toxicant in *R. subnigricans* has been identified as cycloprop-2-ene carboxylic acid (the structural formula is shown below). The authors estimate that consumption of 2–3 fruit bodies may be fatal for humans (Matsuura *et al.*, 2009).

Cycloprop-2-ene carboxylic acid

R. subnigricans belongs to the section *Compactae* Fr. of the genus *Russula* Pers., and this group is characterized by firm flesh which is reddening and then usually blackening, when cut or bruised. The section comprises 6 species in the Nordic countries (Knudsen & Vesterholt, 2012). Three of these 6 species (*R. adusta*, *R. densifolia*, *R. nigricans* and some varieties of *R. anthracina* are described as mild tasting (Knudsen & Vesterholt, 2012). The most common of them in the Nordic countries is Blackening Brittlegill (*R. nigricans*) which is indicated as edible but poor, or inedible

²⁴ Rhabdomyolysis is the breakdown of muscle fibers that leads to the release of muscle fiber contents (myoglobin) into the blood stream. Myoglobin is harmful to the kidney and often causes kidney damage (MedlinePlus Medical Encyclopedia, 2012).

(Egeland & Myhr, 2005; Knudsen & Petersen 2007; Læssøe, 1998; Philips, 2006) or even toxic (Shawn, 1994).

The mild tasting Olive Brittlegill (*R. olivacea* (Schaeff.) Fr.) is generally considered as edible, but some authors warn against this mushroom (Lohmeyer & Künkele, 2006), especially if eaten raw or insufficiently cooked (e.g., Mazza, 2000; Bresinsky & Besl, 1985; Alder, 1954; Pieschl, 1964). However, there are no publications available demonstrating that consumption of thoroughly cooked Olive Brittlegill may give rise to any discomfort.

Pungent/acrid/bitter Brittlegill species are generally considered as inedible or even toxic (Benjamin, 1995; Bresinsky & Besl, 1985; Flammer & Horak, 2003; Roth *et al.*, 1990). There are several intoxication reports describing more or less severe gastrointestinal symptoms after intake of raw or insufficiently cooked pungent species (Beug, 2012; Beug *et al.*, 2006; Flammer & Horak, 2003). The pungent, acrid or bitter constituents are not so well studied as in the related genus Milkcap (*Lactarius*). However, Clericuzio and colleagues have shown, that tasteless velutinal esters, like those occurring in the Milkcap genus, are found in 13 more or less pungent Brittlegill species, also growing in the Nordic countries. Some of these mushrooms were also studied after physical injury, and in several cases formation of the pungent sesquiterpene dialdehydes, velleral and isovelleral, as well as sesquiterpene metabolites of these could be demonstrated (Clericuzio *et al.*, 2008). It is not clear, to what extent pre-treatment before cooking, e.g., parboiling and/or salting destroys the toxicants in pungent Brittlegill species (Benjamin, 1995; Beug, 2012; Bresinsky & Besl, 1985).

The structures of one of the precursor velutinal esters and of the two pungent dialdehydes are shown below:

Stearoylvelutinal

Velleral

Isovelleral

The colouring matters of Brittlegill species are of very different chemical composition. Some of these pigments contain nitrogen, which is the case for the pteridine derivatives riboflavin and the russupteridines, whereas other pigments in this genus, the carotenoids and the sesquiterpenoids do not contain nitrogen. The *Russula*-pigments are not so well studied, especially the blue and violet colours remain to be chemically characterized (for reviews see Eugster, 1973; Gill and Steglich, 1987; Iten *et al.*, 1984; Velisek & Cejpek, 2011). Except for riboflavin and some of the carotenoids, little is known about the biological activity of these pigments.

Green Brittlegill (*Russula aeruginea*) and other glaucous green Brittlegill species are all edible, but can be mistaken for Deathcap (*Amanita phalloides*) which is deadly poisonous and they are therefore not suitable for commercial use. These Brittlegill species are described below under *R. aeruginea*, *R. cyanoxantha*, *R. grisea*, *R. ionochlora*, *R. parazurea* and *R. virescens*.

Database search information

SciFinder by September 2011. Keywords: *Russula*. PubMed by October 2012. Keywords: *Russula*.

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Russula aeruginea Lindblad.

Green Brittlegill (DK: Græsgrøn Skørhat FI: Koivuhapero, IS: Grænhnefla, NO: Grønkremle, SE: Grönkremla).

Background and risk assessment

Green Brittlegill (*Russula aeruginea*) is, as other mild-tasting Brittlegill (*Russula*) species of the section *Heterophyllae* Fr., an edible mushroom. The taste of the young fruit bodies is often slightly acrid (Knudsen & Vesterholt, 2012; Egeland & Myhr, 2003), probably due to the content of certain sesquiterpenes (see *Russula* Pers.).

It forms mycorrhiza with Birch (*Betula*) on acid, sandy or peaty soil and more rarely with Spruce (*Picea*) on more rich soil. It is occasional in the arctic/alpine zone, but otherwise common in the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Green Brittlegill.

Sterner *et al.* (1982) tested an extract of Green Brittlegill in the Ames test using the *Salmonella typhimurium* strains TA98, TA100 and TA2637. The extract was positive in all three strains. The activity was not enhanced in the presence of microsomal enzymes. Unfortunately, Sterner *et al.* (1982) give no information, whether they had controlled for the potential presence of histidine in the extracts. It is well known that false

positives may be obtained in the presence of histidine. Thus, there is no strong indication that extracts of Green Brittlegill are mutagenic.

Recommendation

Green Brittlegill (*Russula aeruginea*) may be mistaken for Deathcap (*Amanita phalloides*), which is deadly poisonous (see Chapter 4). It is therefore not suitable for commercial marketing.

Database search information

SciFinder by September 2011. Keywords: *Russula*. PubMed by October 2012. Keywords: *Russula*.

Listing 3

References

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Russula claroflava Grove (*R. flava* (Romell) Romell)

Yellow Swamp Brittlelegill (DK: Birke-Skørhat FI: Keltahapero, IS: Glóhnefla, NO: Mild gulkremle, SE: Gulkremla).

Background and risk assessment

Yellow Swamp Brittlelegill (*Russula claroflava*) is, as other mild-tasting Brittlelegill (*Russula*) species of the section *Polychromae* Maire, subsection *Integroidinae* (Romagn.) Bon, an edible species (see *Russula* Pers.).

It forms mycorrhiza with Birch (*Betula*) and is rare in Iceland, but otherwise common in the Nordic countries except in the arctic/alpine zone (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Yellow Swamp Brittlelegill.

Recommendation

No special recommendation.

Listing 2

Database search information

SciFinder by September 2011. Keywords: *Russula*. PubMed by October 2012. Keywords: *Russula*.

References

Knudsen H & Vesterholt J (2012): Funga Nordica. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. Nordsvamp: 1–1083.

Russula cyanoxantha (Schaeff.) Fr.

Charcoal Burner (DK: Broget Skørhat FI: No Finnish name, IS: No Icelandic name, NO: Broket kremle, SE: Brokkremla).

Background and risk assessment

Charcoal Burner (*Russula cyanoxantha*) is, as other mild-tasting Brittlegill (*Russula*) species of the section *Heterophyllae* Fr., an edible mushroom (see *Russula* Pers.).

It forms mycorrhiza in deciduous woods. It is very common in the temperate and hemiboreal zones of the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Charcoal Burner.

Recommendation

Green forms of Charcoal Burner (*Russula cyanoxantha*) may be mistaken for Deathcap (*Amanita phalloides*) which is deadly poisonous (see Chapter 4.). Charcoal Burner is therefore not suitable for commercial marketing.

Database search information

SciFinder by September 2011. Keywords: *Russula*. PubMed by October 2012. Keywords: *Russula*.

Listing 3**References**

Knudsen H & Vesterholt J (2012): Funga Nordica. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. Nordsvamp: 1–1083.

Russula decolorans (Fr.) Fr.

Copper Brittlegill (DK: Afblegende Skørhat FI: Kangashapero, IS: No Icelandic name, NO: Gulrød Kremle, SE: Tegelkremla).

Background and risk assessment

Copper Brittlegill (*Russula decolorans*) is, as other mild-tasting Brittlegill (*Russula*) species of the section *Polychromae* Maire, subsection *Integriforminae* Bon and *Melliolentinae* Singer, an edible mushroom (see *Russula* Pers.).

It forms mycorrhiza in coniferous woods, especially with Pine (*Pinus*), but in the subarctic/subalpine zone with Birch (*Betula*). It is rare in Denmark (occasional in Western Jutland), but common in the other Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Copper Brittlegill.

Recommendation

No special recommendation.

Database search information

SciFinder by September 2011. Keywords: *Russula*. PubMed by October 2012. Keywords: *Russula*.

Listing 2

References

Knudsen H & Vesterholt J (2012): Funga Nordica. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. Nordsvamp: 1–1083.

Russula elatior Lindblad

See *Russula paludosa*.

Russula flava (Romell) Romell).

See *Russula claroflava*.

Russula grisea Fr. s. Gillet

No English name (DK: Grålig Skørhat FI: No Finnish name, IS: No Icelandic name, NO: Gråfiolett kremle, SE: Duvkremla).

Background and risk assessment

Russula grisea is, as other mild-tasting Brittlegill (*Russula*) species of the section *Heterophyllae* Fr., an edible mushroom (see *Russula* Pers.)

It forms mycorrhiza in deciduous forests with Beech (*Fagus*) and Oak (*Quercus*). Its distribution in the Nordic countries is unclear due to confusion with related species (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of *R. grisea*.

Recommendation

Russula grisea may be mistaken for Deathcap (*Amanita phalloides*) which is deadly poisonous (see Chapter 4). It is therefore not suitable for commercial marketing.

Database search information

SciFinder by September 2011. Keywords: *Russula*. PubMed by October 2012. Keywords: *Russula*.

Listing 3

References

Knudsen H & Vesterholt J (2012): Funga Nordica. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. Nordsvamp: 1–1083.

Russula integra (L.) Fr. s. Maire (R. polychromae Hora)

Nutty Brittlegill (DK: Mandel-Skørhat, FI: Keltahapero, IS: No Icelandic name, NO: Mandelkremle, SE: Mandelkremla).

Background and risk assessment

Nutty Brittlegill (*Russula integra*) is, as other mild-tasting Brittlegill (*Russula*) species of the section *Polychromae* Maire, subsection *Integroidinae* (Romagn.) Bon, an edible mushroom (see *Russula* Pers.).

It forms mycorrhiza on rich soil in coniferous woods with Spruce (*Picea*) and Pine (*Pinus*) and more rarely in deciduous forests. It is rare in Denmark, but common in hemiboreal and boreal zones of the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Nutty Brittlegill.

Recommendation

No special recommendation.

Database search information

SciFinder by September 2011. Keywords: *Russula*. PubMed by October 2012. Keywords: *Russula*.

Listing 2

References

Knudsen H & Vesterholt J (2012): Funga Nordica. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. Nordsvamp: 1–1083.

Russula ionochlora Romagn.

Oilslick Brittlegill (DK: Violetgrøn Skørhat, FI: Patihapero, IS: No Icelandic name, NO: Iriskremle, SE: Iriskremla).

Background and risk assessment

Oilslick Brittlegill (*Russula ionochlora*) is, as other mild-tasting Brittlegill (*Russula*) species of the section *Heterophyllae* Fr. an edible mushroom. The taste of the gills may be slightly acrid (Knudsen & Vesterholt, 2012), probably due to the content of certain sesquiterpenes (see *Russula* Pers.).

It forms mycorrhiza, mainly with Beech (*Fagus*). It is occasional in temperate and hemiboreal zones of the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Oilslick Brittlegill.

Recommendation

Oilslick Brittlegill (*Russula ionochlora*) may be mistaken for Deathcap (*Amanita phalloides*), which is deadly poisonous (see Chapter 4). It is therefore not suitable for commercial marketing.

Database search information

SciFinder by September 2011. Keywords: *Russula*. PubMed by October 2012. Keywords: *Russula*.

Listing 3**References**

Knudsen H & Vesterholt J (2012): Funga Nordica. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. Nordsvamp: 1–1083.

***Russula obscura* (Romell) Peck**

See *Russula vinosa*.

Russula paludosa Britzelm. (*R. elatior* Lindblad)

("Tall Brittlegill") (DK: Prægtig Skørhat, FI: Isohapero, IS: Myrahefla, NO: Storkremle, SE: Storkremla).

Background and risk assessment

Russula paludosa is, as other mild-tasting Brittlegill (*Russula*) species of the section *Polychromae* Maire, subsection *Integroidinae* (Romagn.) Bon, an edible mushroom. The taste of the young fruit bodies is often slightly acrid (Knudsen & Vesterhold, 2012; Egeland & Myhr, 2003) probably due to its content of velutinal esters (Clericuzio *et al.*, 2008), which by physical injury of the fruit body can be converted to pungent sesquiterpenes (see *Russula* Pers.).

It forms mycorrhiza in coniferous woods on poor soil, especially with Pine (*Pinus*) and in the arctic/alpine zone with Birch (*Betula*). It is common in the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of *R. paludosa*.

Recommendation

No special recommendation.

Database search information

SciFinder by September 2011. Keywords: *Russula*. PubMed by October 2012. Keywords: *Russula*.

Listing 2

References

- Clericuzio M, Gillardoni G, Malagòn O, Vidari G & Finzi PV (2008): Sesquiterpenes of *Lactarius* and *Russula* (Mushrooms): An update. *Natural Product Communications* 3: 951–974.
- Egeland IL & Myhr S (2003): *Norske sopper*. Gyldendal: 1–296.
- Knudsen H & Vesterholt J (2012): *Funga Nordica*. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. *Nordsvamp*: 1–1083.

Russula parazurea Jul. Schäff.

Powdery Brittlegill (DK: Blågrå Skørhat, FI: No Finnish name, IS: No Icelandic name, NO: Blågrønn kremle, SE: Blågrön kremla).

Background and risk assessment

Powdery Brittlegill (*Russula parazurea*) is, as other mild-tasting *Russula* species of the section *Heterophyllae* Fr., an edible mushroom.

Although Powdery Brittlegill may contain velutinal esters (Clericuzio *et al.*, 2008), this does apparently not influence the mild taste of the mushroom (see *Russula* Pers.).

It forms mycorrhiza with various deciduous and coniferous trees on acid soil. It is occasional in Iceland and in the temperate and hemiboreal zones of the other Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Powdery Brittlegill.

Recommendation

Powdery Brittlegill (*Russula parazurea*) may be mistaken for Deathcap (*Amanita phalloides*) which is deadly poisonous (see Chapter 4). It is therefore not suitable for commercial marketing.

Database search information

SciFinder by September 2011. Keywords: *Russula*. PubMed by October 2012. Keywords: *Russula*.

Listing 3**References**

- Clericuzio M, Gillardoni G, Malagòn O, Vidari G & Finzi PV (2008): Sesquiterpenes of *Lactarius* and *Russula* (Mushrooms): An update. *Natural Product Communications* 3: 951–974.
- Knudsen H & Vesterholt J (2012): *Funga Nordica*. Agaricoid, boletoid and cyphelloid genera. *Nordsvamp*: 1–965.

***Russula polychromae* Hora**

See *Russula integra*.

Russula vesca Fr.

The Flirt (Bare-toothed Russula) (DK: Blågrå Skørhat, FI: Palterohapero, IS: Garðhnefla, NO: Nøttekremle, SE: Kantkremle).

Background and risk assessment

The Flirt (*Russula vesca*) is, as other mild-tasting Brittlegill (*Russula*) species of the section *Heterophyllae* Fr., an edible mushroom (see *Russula* Pers.).

It forms mycorrhiza with various deciduous and coniferous trees on acid soil. It is rare in Iceland, but common in temperate and hemiboreal zones and otherwise occasional in the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of The Flirt.

Recommendation

No special recommendation.

Database search information

SciFinder by September 2011. Keywords: *Russula*. PubMed by October 2012. Keywords: *Russula*.

Listing 2**References**

Knudsen H & Vesterholt J (2012): Funga Nordica. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. Nordsvamp: 1–1083.

Russula vinosa Lindblad (*R. obscura* (Romell) Peck)

Darkening Brittlegill (DK: Vinrød Skørhat, FI: Viinihapero, IS: No Icelandic name, NO: Vinrød kremle, SE: Vinkremla).

Background and risk assessment

Darkening Brittlegill is, as other mild-tasting Brittlegill (*Russula*) species of the section *Heterophyllae* Fr., an edible mushroom (see *Russula* Pers.).

It forms mycorrhiza with conifers and in mountains also with Birch (*Betula*). It is occasional in Denmark, but common in the other Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Darkening Brittlegill.

Sterner *et al.* (1982) tested an extract of Darkening Brittlegill in the Ames test using the *Salmonella typhimurium* strains TA98, TA100 and TA2637. The extract was only positive in the strain TA2637. The activity was not enhanced in the presence of microsomal enzymes. Unfortunately, Sterner *et al.* (1982) give no information, whether they had controlled for the potential presence of histidine in the extract. It is well known that false positives may be obtained in the presence of histidine. Thus, there is no strong indication that extracts of Darkening Brittlegill are mutagenic.

Recommendation

No special recommendation.

Database search information

SciFinder by September 2011. Keywords: *Russula*. PubMed by October 2012. Keywords: *Russula*.

Listing 2**References**

- Knudsen H & Vesterholt J (2012): Funga Nordica. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. Nordsvamp: 1–1083.
- Sterner O, Bergman R, Kesler E, Magnusson L, Nilsson B, Wickberg B, Zimerson E & Zetterberg G (1982): Mutagens in larger fungi I. Forty-eight species screened for mutagenic activity in the *Salmonella*/microsome assay. Mutation Research 101: 269–281.

Russula virescens (Schaeff.) Fr.

Greencracked Brittlegill (DK: Spanskgrøn Skørhat, FI: No Finnish name, IS: Bláhnefla, NO: Rutekremle, SE: Rutkremla).

Background and risk assessment

Greencracked Brittlegill is, as other mild-tasting Brittlegill (*Russula*) species of the section *Heterophyllae* Fr., an edible mushroom (see *Russula* Pers.).

It forms mycorrhiza in deciduous and coniferous woods, especially with Beech (*Fagus*). It is rare in Norway, otherwise occasional in temperate and rare in hemiboreal zones of the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Greencracked Brittlegill.

Greencracked Brittlegill has been reported to contain 680 mg/kg (dry weight, 3 samples) of ergothioneine in the fruit bodies (Lee *et al.*, 2009). For further information on ergothioneine: see *Lepista nuda*.

Recommendation

Greencracked Brittlegill (*Russula virescens*) may be mistaken for Deathcap (*Amanita phalloides*) which is deadly poisonous (see Chapter 4). It is therefore not suitable for commercial marketing.

Database search information

SciFinder by September 2011. Keywords: *Russula*. PubMed by October 2012. Keywords: *Russula*.

Listing 3**References**

- Knudsen H & Vesterholt J (2012): Funga Nordica. Agaricoid, boletoid and cyphelloid genera. Nordsvamp: 1–965.
- Lee WY; Park E-J, Jin K & Ka K-H (2009): Ergothioneine contents in fruiting bodies and their enhancement in mycelial cultures by addition of methionine. *Mycobiology* 37: 43–47.

Russula xerampelina (Schaeff.) Fr. s. str

Crab Brittlelegill (Shrimp Mushroom) (DK: Hummer-Skørhat, FI: Sillihapero, IS: Móhneflur, NO: Rød Sildekremle, SE: Sillkremla).

Background and risk assessment

Crab Brittlelegill (*Russula xerampelina*) is, as other mild-tasting Brittlelegill (*Russula*) species of the section *Polychromae* Maire, subsection *Xerampelinae* Singer, an edible mushroom. Although Crab Brittlelegill may contain velutinal esters (Clericuzio *et al.*, 2008), this does apparently not influence the mild taste of the mushroom (see *Russula* Pers.).

It forms mycorrhiza with Pine (*Pinus*) and more rarely with Spruce (*Picea*). It is common in Denmark and the southern parts of the other Nordic countries, but only occasional in the subarctic/subalpine zones (Knudsen & Vesterholt, 2012).

There is a group of 7 closely related and similar Brittlelegill species in the Nordic countries, all belonging to the section *Polychromae* Maire, Subsection *Xerampelinae* Singer (*R. clavipes* Velen, *R. faginea* Romagn., *R. favrei* M.M. Moser, *R. graveolens* Romell, *R. pascua* (F. H. Møller & Jul. Schäff.) Kühner, *R. subrubens* (J.E. Lange) Bon, and *R. xerampelina* (Schaeff.) Fr. s. str. They are very changeable in the colours of the cap, but usually with elements of red or brown colour. The mature fruit bod-

ies have a smell of cooked shellfish, and they are all edible with a mild taste (Knudsen & Vesterholt, 2012).

Only *R. xerampelina* (Schaeff.) Fr. s. str (Crab Brittlelegill), which is common in parts of the Nordic countries and sometimes is traded, is dealt with in the following. The other 6 species are less common in the Nordic countries and not of interest for trade.

There are no intoxications reported after consumption of *R. xerampelina* (Schaeff.) Fr. s. str (Crab Brittlelegill) or the other six related species mentioned above.

Sterner *et al.* (1982) tested an extract of Crab Brittlelegill in the Ames test using the *Salmonella typhimurium* strains TA98, TA100 and TA2637. The extract was positive in the strain TA100. The activity was not enhanced in the presence of microsomal enzymes. Unfortunately, Sterner *et al.* (1982) give no information, whether they had controlled for the potential presence of histidine in the extract. It is well known that false positives may be obtained in the presence of histidine. Thus, there is no strong indication that extracts of the Crab Brittlelegill are mutagenic.

Recommendation

No special recommendation.

Database search information

SciFinder by September 2011. Keywords: *Russula*. PubMed by October 2012. Keywords: *Russula*.

Listing 2

References

- Clericuzio M, Gillardoni G, Malagòn O, Vidari G & Finzi PV (2008): Sesquiterpenes of *Lactarius* and *Russula* (Mushrooms): An update. *Natural Product Communications* 3: 951–974.
- Knudsen H & Vesterholt J (2012): *Funga Nordica*. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. *Nordsvamp*: 1–1083.
- Sterner O, Bergman R, Kesler E, Magnusson L, Nilsson B, Wickberg B, Zimerson E & Zetterberg G (1982): Mutagens in larger fungi I. Forty-eight species screened for mutagenic activity in the *Salmonella*/microsome assay. *Mutation Research* 101: 269–281.

Sparassis crispa (Wulfen) Fr.

Wood Cauliflower (DK: Blomkålssvamp, FI: Kurttusieni, IS: Blómkálssveppur, NO: Blomkålsopp, SE: Blomkålssvamp).

Background and risk assessment

Wood Cauliflower (*Sparassis crispa*) is an edible mushroom.

It is parasitic on roots of conifers, especially Pine (*Pinus*) or saprotrophic on dead and dying coniferous stumps and standing trees. It is occasional in Denmark and in southern parts of Finland, Norway and Sweden but more rare in northern parts, especially in the boreal areas of the Nordic countries (Hansen & Knudsen, 1997). Wood Cauliflower is also cultivated, especially in South-East Asia.

There are no intoxications reported after consumption of Wood Cauliflower, and no natural toxicant constituting a risk for humans has been identified in the mushroom.

Wood Cauliflower contains, besides β -glucans, several types of potentially bioactive constituents, such as phenylpyrrolidines, chalcones, sesquiterpenoids, substituted benzoates and tetrahydrofurans (Chandrasekaran *et al.*, 2011; Jiang *et al.*, 2009; Kawagishi *et al.*, 2007, 2008; Kodani *et al.*, 2009), and ergothioneine (Lee *et al.*, 2009). Compounds of these types have often been shown to have biological activity in experimental studies:

Bioactive constituents

Substituted benzoates: Three substituted benzoates have been isolated from fruit bodies harvested from submerged cultures of the mushroom (Woodward *et al.*, 1993) and they have all been shown to have antifungal activity in *in vitro* studies (Chandrasekaran *et al.*, 2011). The structural formula of one of these benzoates, sparassol, is shown below.

Sparassol

Phenylpyrrolidines: Kawagashi *et al.* (2007, 2008) have isolated a phenylpyrrolidindione derivative and an α,β -unsaturated ketone tautomer of this dione from fruit bodies of Wood Cauliflower in small amounts, 0.8 and 0.4 mg per kg dry weight, respectively. At appropriate concentrations both compounds inhibited melanin synthesis in B16 mouse melanoma cells and growth of methilin-resistant *Staphylococcus aureus*. The α,β -unsaturated ketone-structure is considered to be a structural alert for genotoxicity (EFSA, 2007). The structural formula of the α, β -unsaturated ketone is shown below.

Phenylpyrrolidinedione derivative (enol tautomer)

Flavonoids: Small quantities of the chalcones xanthoangelol and 4-hydroxyderricin have been isolated from Wood Cauliflower (0.1–0.2 mg per kg dried fruit bodies). Both compounds have been demonstrated to be cytotoxic at low doses in human neuroblastoma cell lines cultured *in vitro* (Nishimura *et al.*, 2007).

When given in high concentrations (daily doses of 50 and 100 mg/kg) both compounds inhibit tumour growth in tumour-bearing mice (Kimura & Baba, 2003; Kimura *et al.*, 2004) and growth of methilin-resistant *Staphylococcus aureus in vitro* (Kodani *et al.*, 2008). In addition the flavanone naringenin has been isolated from the mushroom at 35 mg/kg dry weight, corresponding to approximately 4 mg/kg fresh weight (Kim *et al.*, 2008). Naringenin is weakly estrogenic *in vitro*, but this activity has not been confirmed in *in vivo* studies (EFSA, 2010). The structural formula of one of the chalcones, xanthoangelol, is shown below.

Xanthoangelol

Ergothioneine: Lee *et al.* (2009) reported relatively high amounts, 2370 mg/kg (dry weight, 3 samples), of ergothioneine in fruit bodies of Wood Cauliflower. For further information on ergothioneine: see *Lepista nuda*.

Mutagens: Sterner *et al.* (1982) tested an extract of Wood Cauliflower in the Ames test using the *Salmonella typhimurium* strains TA98, TA100 and TA2637. The extract was positive in all three strains. The activity was not enhanced in the presence of microsomal enzymes. Unfortunately, Sterner *et al.* (1982) give no information, whether they had controlled for the potential presence of histidine in the extract. It is well known that false positives may be obtained in the presence of histidine. Thus, there is no strong indication that extracts of Wood Cauliflower are mutagenic.

Recommendation

No special recommendation.

Listing 2

Database search information

SciFinder by September 2011. Keywords: *Sparassis*. PubMed by December 2012. Keywords: *Sparassis*.

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Suillus granulatus (L.) Roussel

Weeping Bolete (Granulated Bolete) (DK: Kornet Rørhat, FI: Jyvästatti, IS: Kornasúlungur, NO: Ringløs smørsopp, SE: Grynsopp).

Background and risk assessment

Weeping Bolete (*Suillus granulatus*) is an edible mushroom.

It grows mycorrhizal with Pine (*Pinus*) in forests and gardens. It is occasional in Denmark and also occasional in temperate-boreal zones, but rare in subalpine zones of the Nordic countries (Knudsen & Vestersholt, 2012).

There are no intoxications reported after consumption of Weeping Bolete, and no natural toxicant constituting a risk for humans has been identified in the mushroom.

Most *Suillus* species contain colouring matters and precursors for these, which mainly belong to three groups of phenolic compounds: the pulvinic acid group (e.g. variegatic acid), the grevillins/pyrandiones (e.g. grevillin D) and the prenylated phenols (e.g. suillin) (Besl & Bresinsky, 1997). Several compounds from these chemical groups have been shown to have antioxidative, antibacterial and antiviral activity or to have inhibitory effects on cytochrome P450 activity, but mainly in *in vitro* stud-

ies (e.g. Geraci *et al.*, 1992; Hayashi *et al.*, 1979; Huang *et al.*, 2009; Liu *et al.*, 2007; Tringali *et al.*, 1989a; 1989b).

Thus, tetraprenylated phenolic constituents have been identified in Weeping Bolete, collected in Germany and Italy, with suillin as the most abundant compound (Jägers *et al.*, 1986, Tringali *et al.*, 1989a). Suillin is mainly found in the viscid skin of the cap and corresponds to about 0.1% of the skin, but the content may vary between 0.01 and 0.5% depending on age and condition of the mushroom (Jägers *et al.*, 1989). Also phenolic pyrandiones (grevillin B, C and D) have been identified in the mushroom, collected in Austria and Germany (Besl *et al.*, 1974; Besl & Bresinsky, 1997). The structural formulae of representatives for these phenols, suillin and grevillin B are shown below.

According to Dong *et al.* (2007) Weeping Bolete (probably collected in China) does also contain the hydroxymethylfuryl- β -carboline-carboxylic acid, flazin, an alkaloid with antiviral properties indicated in *in vitro* studies (Wang *et al.*, 2007; Dong *et al.*, 2007). Moreover, Kang *et al.* (2001) have isolated 1,3,5-trihydroxy-7-methyl-antraquinone from the mushroom (probably collected in Korea).

Ergothioneine: Lee *et al.* (2009) reported relatively small amounts, 90 mg/kg (dry weight, 3 samples), of ergothioneine in fruit bodies of Weeping Bolete. For further information on ergothioneine: see *Lepista nuda*.

It is not possible to evaluate whether the above-mentioned bioactive compounds in Weeping Bolete may have any beneficial or adverse effect in humans consuming this mushroom, as representatives of these constituents have mainly been studied *in vitro*, and as the quantitative occurrence of these constituents is generally not well described.

Recommendation

No special recommendation.

Listing 2

Database search information

SciFinder by July 2012. Keywords: *Suillus*. PubMed by January 2012. Keyword: *Suillus*.

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Suillus grevillei (Klotzsch) Singer

Larch Bolete (Greville's Bolete) (DK: Lærke-Rørhat, FI: Lehtikuusentatti, IS: Lerkisúlungur, Lerkisveppur, NO: Lerkesopp, SE: Lärksopp).

Background and risk assessment

Larch Bolete (*Suillus grevillei*) is an edible mushroom.

It is mycorrhizal with Larch (*Larix*) in plantations, gardens and parks. It is very rarely associated with *Pseudotsuga*. It is common in Denmark and southern parts of the Nordic countries and occasional in middle boreal-subalpine areas (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Larch Bolete, and no natural toxicant constituting a risk for humans has been identified in the mushroom.

Most *Suillus* species contain colouring matters and precursors for these, which mainly belong to three groups of phenolic compounds: the pulvinic acid group (e.g. variegatic acid), the grevillins/pyrandiones (e.g. grevillin D) and the prenylated phenols (e.g. suillin) (Besl & Bresinsky, 1997). Several compounds from these chemical groups have been shown to have antioxidative, antibacterial and antiviral activity or to have inhibitory effects on cytochrome P450 activity, but mainly in *in vitro* stud-

ies (e.g. Geraci *et al.*, 1992; Hayashi *et al.*, 1979; Huang *et al.*, 2009; Liu *et al.*, 2007; Tringali *et al.*, 1989a; 1989b).

Thus, Larch Bolete contains representatives from all these phenolic groups; from the grevillin/pyrandonone group, grevillin A, B and C (Gill & Kiefel, 1988; Massow & Huber, 1977; Steglich *et al.*, 1972), from the tetraprenylated phenol group, e.g. bolegreviol, an isomer of suillin (Hayashi *et al.*, 1989) and from the pulvinic acid group, varieagatic acid (Besl & Bresensky, 1997). Additionally, some terphenylquinones, thelephoric acid and cyclovariegatin have been identified in Larch Bolete (Edwards & Gill, 1973; 1975). The structural formulae of representatives of these phenols, grevillin B, bolegreviol, varieagatic acid and the terphenyl quinone, thelephoric acid, are shown below.

Grevillin B

Bolegreviol

Thelephoric acid

Variegatic acid

It is not possible to evaluate whether the above-mentioned bioactive compounds in Larch Bolete may have any beneficial or adverse effect in humans consuming this mushroom, as representatives of these constituents have mainly been studied *in vitro*, and as the quantitative occurrence of these constituents is generally not well described.

Recommendation

No special recommendation.

Listing 2

Database search information

SciFinder by July 2012. Keywords: *Suillus*. PubMed by January 2012. Keywords: *Suillus*.

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Suillus luteus (L.) Roussel

Slippery Jack (DK: Brungul Rørhat, FI: Voitatti, IS: Furusúlungur, Furu-sveppur, NO: Smørsopp, SE: Smörsopp).

Background and risk assessment

Slippery Jack (*Suillus luteus*) is an edible mushroom. It is mostly used as fresh, but is also commonly traded, especially dried, often in mixtures of mushrooms.

It is mycorrhizal with pine (*Pinus*) and is very common in the Nordic countries in temperate–coniferous subalpine areas (Knudsen & Vesterholt, 2012).

Although Slippery Jack generally is regarded as safe to eat and is commonly traded, it has given rise to some intoxications and e.g., Lincoff (2010) recommends to remove the slime (the glutinous skin of the cap) before its use for consumption.

Human observations

Bobrowski (1966) described a person who experienced intoxications in 1958 and 1964 with fatigue, feeling unwell and with temporary icterus after consumption of Slippery Jack. The symptoms disappeared spontaneously after a short time. In 1965, the same person (30 years old woman) had a third intoxication after consumption of the same mushroom

species. The poisoning was diagnosed as severe acute haemolysis with kidney insufficiency. After one month of hospitalization, the patient was discharged without symptoms. Other family members did eat the same mushroom dish without any adverse reactions.

In 1984, a case of mild poisoning after consumption of Slippery Jack was reported (Prager & Goos, 1984). The involved person, a 31 year old, healthy man had eaten the mushroom before, after peeling of the glutinous cuticle, without illness. This time he had eaten newly-emerged fruit bodies which were in excellent condition, and the cuticle of the caps was not at all slimy and therefore not removed. He ate about 300 mL of the mushroom, which was sliced and cooked with butter and lemon. The remaining mushrooms were sliced and dried. Watery diarrhoea commenced 15 minutes after consumption of the dish. After five days, he had totally recovered. He had no nausea, pain or other symptoms except for accompanying fatigue. Six days later he made a soup with about 3 g of the sliced, dried mushrooms. He ate about 3 slices of the mushroom with the soup. About 15 minutes later diarrhoea sets in and lasted for 5 days. The remaining soup was refrigerated. Eight days after the meal with the soup as an experiment, he heated the frozen soup and ate some of it, but without mushroom slices and had no symptoms. Next day he ate 2 slices of the mushroom from the soup, and again experienced the symptoms he had obtained earlier. Apparently, the cuticle contained non-volatile toxicant(s) stable to heating and drying and not readily extracted from the mushroom slices by boiling, and seems rather potent in this individual (Prager & Groos, 1984). The identity of the consumed Slippery Jack was confirmed by field characteristics and microscopy.

Beug *et al.* (2006) have summarized thirty-plus years of mushroom poisonings in the USA from approximately 2,000 reports, covering data until the end of 2005. In this period totally 8 cases (6 adults and 2 children) from North America are registered in the summary. Seven of the cases with symptoms after consumption of Slippery Jack included gastrointestinal distress.

Since the report by Bobrowski in 1966, there are no published data available on poisonings in Europe with this commonly consumed mushroom.

Although the potential toxicant is unknown, several anticipated bioactive constituents occur in Slippery Jack.

Bioactive constituents

Colouring matters: It contains colouring matters and precursors for these, which mainly belong to three groups of phenolic compounds: the pulvinic acid group (e.g. variegatic acid), the grevillins/pyrandiones (e.g.

grevillin D) and the prenylated phenols (e.g. suillin) (Besl & Bresinsky, 1997). Several compounds from these chemical groups have been shown to have antioxidative, antibacterial and antiviral activity or to have inhibitory effects on cytochrome P450 activity, but almost only in *in vitro* studies (e.g. Geraci *et al.*, 1992; Hayashi *et al.*, 1979; Huang *et al.*, 2009; Liu *et al.*, 2007; Tringali *et al.*, 1989a; 1989b).

At least three different pulvinic acid derivatives have been identified in Slippery Jack, atromentic acid, variegatic acid and xerocomic acid (Besl & Bresinsky, 1997), four grevillins, grevillin A, B C and D (Besl *et al.*, 1974; Besl & Bresinsky, 1997) and the tetraprenylated phenol suillin (Jägers *et al.*, 1986).

The structural formulae of variegatic acid, grevillin B and suillin, representatives for the three groups of phenols in Slippery Jack, are shown below.

Grevillin B

Suillin

Variegatic acid

Indole compounds: Muszynska *et al.* (2011) have identified several indole compounds in Slippery Jack, but at low levels corresponding to approximately 1 mg/kg (fresh weight) or lower, except for serotonin found at a level of approximately 34 mg/kg (fresh weight). However, serotonin is apparently completely destroyed during cooking (Muszynska *et al.*, 2012).

Ergothioneine: Lee *et al.* (2009) reported relatively high amounts, 2270 mg/kg (dry weight, 3 samples), of ergothioneine in fruit bodies of Slippery Jack. For further information on ergothioneine: see *Lepista nuda*.

Mutagens: Morales *et al.* (1990a; 1990b) studied mushroom extracts in the Ames test. Extracts of freshly harvested and frozen Slippery Jack mushrooms were reported to be weakly mutagenic in the *Salmonella* strains TA98 and TA100 (for the fresh mushroom less than doubling in mutation frequency). Extracts of canned Slippery Jack were negative both in the Ames test and in the Chinese Hamster Ovary (CHO/HPRT) cell mutation assay. Apparently, Morales *et al.* (1990a, 1990b) did not control for possible histidine in the extracts for the Ames test. Thus, there is no strong indication that extracts of the Slippery Jack are mutagenic.

It is not possible to evaluate whether the above-mentioned bioactive compounds in Weeping Bolete may have any beneficial or adverse effect in humans consuming this mushroom, as representatives of these constituents have mainly been studied *in vitro*, and as the quantitative occurrence of these constituents is generally not well described.

Recommendation

No special recommendation.

Listing 1

Database search information

SciFinder by July 2012. Keywords: *Suillus*. PubMed January 2012. Keywords: *Suillus*.

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Suillus variegatus (Sw.) Kuntze

Velvet Bolete (Variegated Bolete) “Swedish Jack” (DK: Broget Rørhat, FI: Kangastatti, IS: Sandsúlungur, NO: Sandsopp, SE: Sandsopp).

Background and risk assessment

Velvet Bolete (*Suillus variegatus*) is an edible mushroom.

It is mycorrhizal with pine (*Pinus*) in forests and plantations. It is very common in Denmark and in temperate-coniferous subalpine areas of the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Velvet Bolete, and no natural toxicant constituting a risk for humans has been identified in the mushroom.

Most *Suillus* species contain colouring matters and precursors for these, which mainly belong to three groups of phenolic compounds: the pulvinic acid group (e.g. variegatic acid), the grevillins/pyrandiones (e.g. grevillin D) and the prenylated phenols (e.g. suillin) (Besl & Bresinsky, 1997). Several compounds from these chemical groups have been shown to have antioxidative, antibacterial and antiviral activity or to have inhibitory effects on cytochrome P450 activity, but mainly in *in vitro* studies (e.g. Geraci *et al.*, 1992; Hayashi *et al.*, 1979; Huang *et al.*, 2009; Liu *et al.*, 2007; Tringali *et al.*, 1989a; 1989b).

Thus, Velvet Bolete contains the two pulvinic acid derivatives: variegatic acid and atromentic acid and the related lactone variegatorubin (Edwards and Elseworthy, 1967; Beaumont *et al.*, 1968; Bresinsky & Orendi, 1970) and the prenylated phenol suillin (Jägers *et al.*, 1986), but apparently no grevillins.

The structural formulae of suillin and variegatic acid, as representatives for the two groups of phenolic constituents in the mushroom are shown below.

Variegatic acid

Suillin

It is not possible to evaluate whether the above-mentioned bioactive compounds in Velvet Bolete may have any beneficial or adverse effect in humans consuming this mushroom, as representatives of these constituents have mainly been studied *in vitro*, and as the quantitative occurrence of these constituents is generally not well described.

Recommendation

No special recommendation.

Listing 2

Database search information

SciFinder by July 2012. Keywords: *Suillus*. PubMed January 2012. Keywords: *Suillus*.

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Tricholoma auratum (Paulet) Gillet

See *Tricholoma equestre*.

Tricholoma equestre (L.) P. Kumm. (*T. flavovirens* (Pers.) S. Lundell, *T. auratum* (Paulet) Gillet)

Yellow Knight (Man on Horseback, “Canary Mushroom”) (DK: Ægte Ridderhat, FI: Kangaskeltavalmuska, IS: Riddaraskjalda, NO: Riddermusserong, SE: Riddarmusseron).

Background and hazard identification

Worldwide, Yellow Knight has for many years been regarded as a very good edible mushroom. However, after Bedry *et al.* (2001) in France, and Chodorowski *et al.* (2002; 2003), Sein Anand *et al.* (2009), and Sein Anand & Chwaluk (2001; 2010) in Poland, reported on serious intoxications after consumption of this mushroom, its edibility has been questioned. However, Nylén (2012) has recently claimed that *T. equestre* is an excellent food mushroom, and that it is the related look-alike *T. auratum* which previously has been considered edible, which has been responsible for the intoxications reported in France and Poland.

Yellow Knight (*Tricholoma equestre*) forms commonly mycorrhiza with Pine (*Pinus*) in sandy, poor nutrient soil or more rarely on calcareous soil in the temperate – boreal zones of the Nordic countries (Knudsen & Vesterholt, 2012). Other forms (or possibly species) of Yellow Knight grow under deciduous trees, especially Aspen (*Populus tremula*)

and Poplar (*Populus*) or under Spruce (*Picea*). Taxonomically, Yellow Knight (*T. equestre*/*T. flavovirens*/*T. auratum*) is apparently a group of related species, which requires genetic analysis to be distinguished. Hitherto, some authors have treated the Yellow Knight complex as one species, e.g. Knudsen & Vesterholt (2008), other authors as more than one species, e.g. Nylén (2012).

Hazard characterisation

Human intoxications: Since 1992, 12 cases of delayed rhabdomyolysis²⁵ have been documented in France after ingestion of several consecutive meals including large quantities of Yellow Knight. Three of the 12 cases were fatal. The 12 cases clearly implicated Yellow Knight as the cause of rhabdomyolysis. No other potential cause of rhabdomyolysis like direct injury, very hard exercise, infections, certain medications, immunotoxicity or toxin exposure were found. The consumed Yellow Knight leading to the intoxications was collected under Pine on a sandy coast in south-western France and had been positively identified (Bedry *et al.*, 2001; Bedry & Gromb, 2009).

Five Polish cases, including one boy (5 years) and 4 adults (2 men, aged 20 and 72 years and two women, aged 42 and 45 years) were hospitalized after having consumed large quantities of Yellow Knight (100–400 g) daily for several (3–10) consecutive meals.

The child was admitted to hospital in deep coma with cyanosis and convulsions, 4 hours after the last meal (300–400 g of the mushroom daily during 4 consecutive days). After 2–3 weeks of supportive treatment at the hospital, all his rhabdomyolysis symptoms with complications had disappeared (Chodorowski *et al.*, 2003; Sein Anand *et al.*, 2009)

The 72 years old man was admitted to hospital with muscle weakness and myalgia one day after the last of ten consecutive mushroom meals (300–400 g mushroom). He complained about dyspnoea and breathing problems the second day of hospitalisation, and shortly after, irrespective of intubation and connection to respirator, he died by cardiac arrest (Sein Anand *et al.*, 2009; Sein Anand & Chwaluk, 2010).

The 42-years old mother and her 20 years old son were admitted to hospital two days after the last of 9 consecutive meals with Yellow

²⁵ Rhabdomyolysis is the breakdown of muscle fibers that leads to the release of muscle fiber contents (myoglobin) into the blood stream. Myoglobin is harmful to the kidney and often causes kidney damage (MedlinePlus Medical Encyclopedia, 2012).

Knight, each with about 100–300 g mushroom. Their symptoms included fatigue, muscle weakness, myalgia, loss of appetite, nausea and sweating. All symptoms disappeared within 3 weeks of supportive treatment, but the muscle weakness lasted for another 2–3 months (Chorodowski *et al.*, 2002; Sein Anand *et al.*, 2009; Sein Anand & Chwaluk, 2010).

The 45 years old woman was admitted to hospital approximately 5 days after the last of three meals consumed on three consecutive days, each meal with about 2–300 g of the mushroom, because of fatigue, myalgia and profuse sweating. All symptoms had disappeared after 10 days, except that the serum creatine kinase was still increased when she left hospital the eleventh day.

There was no indication that any of the Polish patients were exposed to parasites, microorganisms or systemic disease (Chodorowski *et al.*, 2002; 2003; Sein Anand *et al.*, 2009; Sein Anand & Chwaluk, 2010).

Sein Anand & Chwaluk (2010) reviewed the information on all patients above admitted to the poison centres in Gdansk and Bila Podlaska in the years from 2001 to 2010, who had been poisoned with Yellow Knight. The symptoms included muscle weakness, nausea (without vomiting), diaphoresis (without fever) and in most serious cases acute respiratory failure. There was a marked increase in serum/plasma levels of creatine kinase, and aspartate and alanine aminotransferase. The mortality rate was 20%. Apparently, the severity of the intoxications was correlated with the amounts of Yellow Knight consumed. The most serious effects were acute respiratory failure, myocarditis with cardiac arrhythmia and cardiovascular collapse. It was emphasized that the clinical picture might be different for children, e.g. the creatine kinase and alanine aminotransferase were much less increased compared to the adults and did not correspond well with the grave clinical conditions (Sein Anand & Chwaluk, 2010).

It has also been speculated, whether an intoxication in Finland was caused by Yellow Knight (Nieminen *et al.*, 2009).

Horn *et al.* (2005) described in a poster a 71 years old man in Germany, who had eaten Yellow Knight in large quantities for many years. He was admitted to hospital six days after having consumed Yellow Knight meals twice daily on six consecutive days and showed typical symptoms of rhabdomyolysis, myalgia, fatigue, muscle weakness and profuse sweating. The first day after admission to the hospital the patient's creatine kinase, myoglobin, and aspartate and alanine aminotransferase were significantly increased. However, simvastatin treatment for hyperlipidemia had been initiated 6 months before the admission to hospital. The treatment at the hospital was supportive and the

simvastatin treatment was discontinued. As a result the symptoms disappeared and the laboratory findings decreased, but they were not fully normalised 10 days later.

It is not clear in this case, whether the cause of the rhabdomyolysis was the simvastatin exposure alone (less than 0.1% of patients treated with simvastatin may get myopathy and/or rhabdomyolysis), Yellow Knight alone, or a combination of both (Horn *et al.*, 2005).

Although a study by Chodorowsky *et al.* (2005) could not demonstrate toxic effects in patients taking statins and consuming large quantities of Yellow Knight (between 300 and 1,200 g for 4 consecutive days), Horn *et al.* (2005) discourage patients receiving statins to consume Yellow Knight.

Recently Chwaluk (2013) reported a case where a 57-year old man was hospitalized with severe pain and weakness of thigh muscles and high serum creatine kinase activity. Within two days before hospitalisation he had repeatedly consumed large amounts of stewed *Leccinum* and *Boletus* mushrooms. The man recovered after several days of hospitalisation.

There are no information on which quantities and species of *Leccinum* and *Boletus* the man had consumed. This is apparently the first report, where consumption of *Leccinum* and/or *Boletus* mushrooms have given rise to clinical signs of toxicity and biochemical changes, indicating rhabdomyolysis.

These cases highlight the problem of identifying the cause of the rhabdomyolysis after consumption of Yellow Knight and possibly other mushrooms.

Experimental studies

In order to demonstrate, that Yellow Knight is likely to be the cause of the rhabdomyolysis observed in consumers of the mushroom, three research teams have tested the hypothesis by supplying mice for several days with different preparations of Yellow Knight: in water, by gavage or mixed into their feed.

In the first of two studies described by Bedry *et al.* (2001), 3 groups of 3 mice were during three days given a total dose of 2, 4 or 6 g Yellow Knight per kg body weight as a powder in water by gavage. The highest total dose given to mice (6 g per kg body weight) corresponds to a 60 kg person consuming totally 3 kg fresh mushroom over the course of 6 meals in a period of 3 days. The treated mice had a dose dependent increase in the serum creatine kinase level measured 2 days after the final Yellow Knight dose. The increase was statistically significant at the two highest total doses (4 and 6 g per kg body weight).

In a second study, Bedry *et al.* (2001) used 5 groups of 5 mice each; four groups being gavaged for 3 days with two different extracts (aqueous or chloroform-methanol extracts) of Yellow Knight or the Oyster Mushroom (*Pleurotus ostreatus*), whereas the fifth group was gavaged with the positive control p-phenylendiamine – a potent myotoxin in mice – (70 mg per kg body weight per day), for 3 days. The total dose of Yellow Knight extract corresponded to a total dose of 6 g per kg mouse. The serum creatine kinase levels were determined 4 days after the final mushroom dose and were significantly increased in the two groups given Yellow Knight, as well as in the p-phenylendiamine group. The Oyster Mushroom extracts had no influence on the serum creatine kinase activity. Thus the results of the two studies in mice supported the involvement of Yellow Knight in the etiology of rhabdomyolysis in humans (Bedry *et al.*, 2001).

In a similar animal feeding study by Chodorowski *et al.* (2004) thirty male mice were divided into 6 groups of 5 males and supplied aqueous or chloroform/methanol extracts of Yellow Knight, the respective solvents, a powder of Yellow Knight, or the positive control p-phenylendiamine. Contrary to the findings of Bedry *et al.* (2001), the Polish investigators observed no effect of the mushroom on the level of creatine kinase. However, as the sample of Yellow Knight mushrooms had been stored for 12 months at -20 °C, the authors concluded that extracts of Yellow Knight stored for 12 months at -20 °C do not cause rhabdomyolysis in mice (Chodorowski *et al.*, 2004).

Also Nieminen and colleagues have fed Yellow Knight (and several other edible mushroom species) to mice (Nieminen *et al.*, 2005, 2006, 2008). In their first study, 42 mice were divided into 7 groups of 6 mice each (Nieminen *et al.*, 2005). Three groups received 3, 6 and 9 g per kg body weight per day of dried, powdered Yellow Knight mixed into pelleted feed, one group received 9 g per kg body weight freshly frozen, then thawed and homogenized Yellow Knight mixed into the feed, one group received 9 g per kg body weight per day of dried powdered Cep (*Boletus edulis*) mixed into feed, one group (positive control) received 75 mg per kg body weight per day of p-phenylendiamine, and one group (control) received the regular mouse feed. Animals were sacrificed after 5 days feeding, blood and muscle samples were taken, and livers and kidneys were dissected and weighed. Body mass, feed and water intake, organ weights and biochemical parameters were measured. In the high dose dried Yellow Knight group the feed intake was reduced, the plasma cholesterol increased (dose-dependently) and the plasma creatine kinase activity significantly increased (the increase was dose dependent) but

plasma creatine kinase was not affected in the group receiving the same amount of frozen/thawed Yellow Knight. However, and surprisingly, creatine kinase was increased also in the group receiving 9 g per kg body weight per day of dried Cep. The plasma aspartate and alanine aminotransferases were unaffected by the exposure (Nieminen *et al.*, 2005).

Together with the initial animal feeding study, the investigators reported a study on 4 healthy human volunteers (2 males and 2 females, age 29–33 years) who consumed 0.15 g dried Yellow Knight in a rice dish. Blood samples were taken before the experiment and 3 and 7 days later. There were no significant changes in the plasma creatine kinase, aspartate or alanine aminotransferase activities (Nieminen *et al.*, 2005).

Overall, the authors concluded that the results confirm that consumption of Yellow Knight may cause elevated creatine kinase activity and that the effect most probably requires high amounts of ingested mushroom, individual susceptibility and repeated meals for many days (Nieminen *et al.*, 2005).

In a second investigation on Yellow Knight, Nieminen *et al.* (2008) focused more on the mechanism of Yellow Knight poisoning by studying subchronic effects of the mushroom on mice at a dose level relevant to human consumption in a 28-day repeated dose toxicity study. Parameters studied included effects on biochemical markers of toxicity and histological findings related to the exposure. The study had two groups of six male mice. The control group received regular mouse feed and the treatment group 12 g per kg body weight per day of Yellow Knight (freshly frozen at -80 °C, thawed, homogenized and mixed into standard feed). After 28 days of treatment, animals were sacrificed and blood and muscle samples taken for analysis, and livers, hearts and kidneys dissected and weighed. There was no effect of the treatment on body mass change and food intake, and absolute relative organ weights. Regarding plasma clinical chemistry, bilirubin was increased, while the concentration of uric acid and protein was reduced. The activities of plasma creatine kinase and plasma creatine kinase-MB (a creatine kinase isoenzyme more specifically related to myocardial injury than creatine kinase) were increased. The histological analysis revealed a higher incidence of inflammation in the pericardial fat of mice supplied Yellow Knight (Nieminen *et al.*, 2008). The investigators concluded that: “Regular consumption of Yellow Knight should not be recommended and that the results support previous findings that the harmful effects probably require prolonged exposure and high amounts of Yellow Knight” (Nieminen *et al.*, 2008; 2009).

As a follow-up on the study by Nieminen *et al.* (2005) showing that not only feeding mice with Yellow Knight but also with Cep (*Boletus edu-*

lis) could give rise to elevated levels of plasma creatine kinase activities and thereby suspected myotoxicity, Nieminen *et al.* (2006) tested several edible wild mushrooms representing four genera (Brittlegill species (*Russula* spp.), Chanterelle (*Cantharellus cibarius*), Forest Lamb (*Albatrellus ovinus*) and Orange Birch Bolete (*Leccinum versipelle*)) in mice. The plasma creatine kinase activity increased with all studied mushrooms at the highest dose of 9 g per kg body weight per day (for *Russula* spp. even at 3 g) in the mice. The results support the hypothesis that the previous observed toxic effects with Yellow Knight are not specific to this mushroom, but probably represent a non-specific response requiring individual sensitivity and a significant amount of ingested mushroom to manifest itself (Nieminen *et al.*, 2006). A similar conclusion was drawn by Chwaluk (2013) based on his abovementioned case report on intoxication after consumption of *Leccinum* and *Boletus* mushrooms.

Some scepticism with respect to the toxicity of *T. equestre*/Yellow Knight has been expressed by the American mycological/toxicological expert Benjamin (2003), and he raises numerous questions to the publication by Bedry *et al.* (2001), e.g., he asks for more complete clinical history, if all who ate the same mushroom meals develop symptoms, did the parents have predispositions to muscle disease, are there geographical/environmental differences and could there be a taxonomical problem? He also raised questions about the experimental data (Benjamin, 2003).

More recent toxicological reviews on mushrooms also include *T. equestre*/Yellow Knight intoxications, which should be interpreted with caution, but with the present knowledge one should refrain from consuming this mushroom (Diaz, 2005; Flammer & Schenk-Jäger, 200; Karlson-Stiber & Persson, 2003; Saviuc & Danel, 2006).

Causative inherent toxicant

The causative toxicant has not been identified. It is indicated that drying of the mushroom does not influence the effect of the toxicant and that the toxicant is soluble both in water and in chloroform-methanol (Nieminen *et al.*, 2005). It is also shown that storage of extracts of Yellow Knight at -20 °C for 12 months apparently reduces the activity of the toxicant(s) as such extracts have been shown to be harmless to mice (Chodorowsky *et al.*, 2004). Some potential bioactive constituents have been identified in Yellow Knight, e.g. Muszynska *et al.* (2009) isolated a series of simple biologically active indole compounds (tryptophan, 5-hydroxytryptophan, serotonin, thryptamine and melatonin). Some of these constituents are tissue hormones and neurotransmitters and high intakes of tryptophan can damage the nervous system (Stone *et al.*, 2003). The yellow pigment flavomannin-6,6-dimethylether (Steglich *et*

al., 1972; Pachón-Peña *et al.*, 2009) is also biologically active, but it is not known to have myotoxic properties.

Few other mushrooms or mushroom constituents are known to have been involved in myopathy and possibly rhabdomyolysis. Lovastatin, which occurs in Oyster Mushroom (*Pleurotus oestratus*) and other edible mushrooms, has as side effects in a little fraction of humans using lovastatin as a drug developed myopathy and possibly rhabdomyolysis. For further information on statins: see *Pleurotus oestratus*.

The Asian Brittlegill *Russula subnigricans* has given rise to several cases of rhabdomyolysis, some with fatal outcome (Lee *et al.*, 2001; Matsuura *et al.*, 2009). In this mushroom the causative inherent toxicant has recently been identified as cycloprop-2-ene carboxylic acid (Matsuura *et al.*, 2009). In order to better understand the mechanism of the rhabdomyolysis after consumption of Yellow Knight, it would be important to identify the toxicant(s) in this mushroom.

Exposure assessment

There are no data on the general consumption of Yellow Knight. However, all reported intoxications have been observed after recent intake of several, consecutive meals with large quantities of Yellow Knight, and where more specified, between 100 and 400 g per meal for 3 to 10 consecutive days (Bedry *et al.*, 2001; Chodorowski *et al.*, 2002; 2003; Sein Anand & Chwaluk, 2010; Sein Anand *et al.*, 2009).

Risk characterisation

Yellow Knight (*T. equestre*) has been involved in at least 17 cases of myopathy/rhabdomyolysis, of which four were fatal. Five studies in mice have supported that Yellow Knight can be the cause, as feeding with Yellow Knight could give rise to dose-dependent increase in creatine kinase activities in these animals.

Uncertainties: It should be emphasized that the rhabdomyolysis in humans are only reported after consumption of high amounts of Yellow Knight in several consecutive meals. It should also be noted that the number of animals in each group in the experimental studies was low 3–6 animals per group and only one sex in each study.

The causative inherent toxicant(s) is(are) not identified and the taxonomy of the Yellow Knight complex *T.equestre*/*T. flavovirens*/*T. auratum* needs to be clarified.

Recommendation

Severe, also some fatal intoxications have been reported after consumption of repeated meals with substantial amounts of Yellow Knight (*Tricholoma equestre*).

Yellow Knight should not be marketed before a safe level of use can be established. This requires identification of the toxicant(s), knowledge of its concentration in the mushroom and clarification of the mechanism of toxicity.

Due to the intoxications, some European countries have forbidden trade with Yellow Knight and other countries have given warnings.

Listing 4

Database search information

SciFinder by February 2012. Keywords: *Tricholoma equestre* or *flavovirens* or *auratum*. Rhabdomyolysis and mushrooms.

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Tricholoma flavovirens (Pers.) S. Lundell

See *Tricholoma equestre*.

Tricholoma gambosum (Fr.) P. Kumm.

See *Calocybe gambosa*.

Tricholoma georgii (L.) Quél.

See *Calocybe gambosa*.

Tricholoma matsutake (S. Ito & S. Imai) Singer
(*T. nauseosum* (A. Blytt) Kytöv)

Matsutake (DK: Duft-Ridderhat ("Matsutake"), FI: Tuoksuvalmuska, IS: No Icelandic name, NO: Kransmusserong, SE: Goliatmusseron ("Matsutake")).

Background and risk assessment

Matsutake (*Tricholoma matsutake*) is a highly appreciated edible mushroom, which is especially consumed in parts of Asia.

Matsutake grows mycorrhizal with Pine (*Pinus*) on sandy soils and rocky habitats. It is rare, but locally occasional in hemiboreal/boreal/subalpine/subarctic zones of the Nordic countries. It is very rare in the temperate zone (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Matsutake, except for some cases of allergenicity and possibly other types of hypersensitivity. Allergens and other bioactive constituents are described below.

Bioactive constituents

Allergens: In some of the cases described below the allergens are not characterized.

The first report, that ingestion of Matsutake may lead to anaphylaxis, appeared at the end of the 1990s. Okano and Sakuma (1997) reported a

case of food-dependent exercise-induced anaphylaxis (pruritus, diffuse erythematous rashes, weals and oedema, abdominal distress, diarrhoea and extreme weakness) in a 17-year old healthy male high-school basketball player who had ingested Matsutake mushrooms a few hours before athletic exercise. The requirement of both components was confirmed in provocation tests. The observation of allergic reactions may be dependent on physical exercise had previously been observed after intake of shellfish, squid and octopus, celery and wheat, peach, grape, kiwi, hazelnut, egg and milk, but not with a mushroom.

It has subsequently become clear that physical exercise is not a prerequisite for anaphylactic reaction after ingestion of Matsutake. For example an 8-year old girl was taken to hospital one hour after consuming around 14 g of the mushroom in a restaurant. She experienced nausea, swelling of her face and dyspnea, difficulties to breathe and severe stridor. Angioderma of her face, eyelids and fingers, and skin erythema were also evident (Ichikawa *et al.*, 2006). Skin-prick tests were positive against a Matsutake extract and IgE antibodies were detected in the patient's serum but not in sera from healthy controls. Cytokines and chemical mediators of immunological reactions were significantly higher at the time of the anaphylaxis.

Another case showing anaphylactic reactions to Matsutake was challenged with about 100 g grilled Matsutake to confirm the allergic reaction. Within half an hour the provocation resulted in throat irritation, dyspnea and reduced peak flow rate. All these symptoms disappeared spontaneously 4 hours later but after another hour the patient suffered from dyspnea and general cutaneous erythema, with urticaria of his face and body. Medical treatment had to be initiated. Whereas a skin prick test was positive for Matsutake, no mushroom specific IgE could be identified in the patient serum (Komase *et al.*, 1999). This is not the only case in which anaphylaxis has occurred, but no IgE has been identified.

A patient with a history of atopic asthma but no history of food allergic reactions experienced anaphylactic reactions consisting of systemic urticaria, dyspnea and consciousness disturbances 10 minutes after ingesting a soup containing several vegetables and Matsutake (Toda *et al.*, 2010). One year after recovery, the woman had another anaphylactic reaction consisting of systemic urticaria and abdominal pain after consuming baked Matsutake. It was not until after this episode, the mushroom was incriminated as the cause. The suspicion was confirmed by a positive skin prick test with Matsutake extract and a positive basophil histamine release test.

At the end of 2010 about 13 cases of anaphylaxis caused by Matsutake have been described in Japan (Amano *et al.*, 2003; Ishikawa *et al.*, 2002, 2006; Komase *et al.*, 1999; Okano & Sakuma, 1997; Takamasu *et al.*, 2000; Terao *et al.*, 1984; Toda *et al.*, 2010).

A Japanese study analysed the association between allergic reactions to one type of food and allergic reactions to other types of food (Kana-gawa *et al.*, 2009). This study was based on 878 families with 1,383 allergic patients, including 402 with anaphylaxis. In total these patients described 6,549 food allergies. The analysis showed that the occurrence probability of an allergy combination Matsutake – milk was high. Based on this finding, the investigators speculated that Matsutake does not independently induce food allergies. However, there is no experimental support for this hypothesis.

Cyanogenic compounds: Matsutake may contain small amounts of cyanogenic compounds. In seven samples of the raw mushroom from Korea, Japan and Canada, levels up to 0.8 mg/kg (average 0.2 mg) of hydrogen cyanide have been demonstrated (Shindo *et al.*, 1999). Such low levels are of no health concern in relation to consumption of Matsutake.

Sesquiterpenoids: The sesquiterpene farnesa-1,6-diene-3,10,11-triol, an α,β -unsaturated secondary alcohol, has been isolated from the fruit bodies of Matsutake (Ohnuma *et al.*, 2000). When the mushroom is consumed, this compound may be oxidized to a potential bioactive α,β -unsaturated ketone. The α , β -unsaturated aldehyde and ketone structures are generally considered as structural alerts for genotoxicity (EFSA, 2007).

Ergothioneine: Matsutake has been reported to contain 740 mg/kg (dry weight, 3 samples) of ergothioneine in the fruit bodies (Lee *et al.*, 2009). For further information on ergothioneine: see *Lepista nuda*.

Experimental studies: A preparation derived from cultured mycelia of Matsutake was studied for mutagenic activity in bacterial bioassays. The preparation neither induced gene mutations in four different strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and one strain of *Escherichia coli* in the absence of a metabolic activation system, nor in the presence of such a system (Torigoe *et al.*, 2003). The same preparation used for the mutagenicity tests was used for three experimental studies on rats, one acute toxicity study, one 28-day toxicity study and one 90-day sub-chronic feeding study.

The acute rat study identified the oral LD₅₀ to be higher than 2,000 mg/kg body weight in both male and female animals (Yamamoto *et al.*, 2003a). Neither in the 28-day toxicity study, nor in the 90-day subchronic study, oral administration of the preparation had any dose-related effects on studied parameters (e.g. body weight, clinical chemis-

try, organ weights and histopathology). The no observed adverse effect level (NOAEL) in both males and females were thus higher than the highest dose tested in the studies, that is higher than 5,000 mg of the preparation/kg body weight in the 28-day study, and higher than 10,000 mg of the preparation/kg body weight in the 90-day study (Omori *et al.*, 2003; Yamamoto *et al.*, 2003b).

All the experimental studies have been performed with a preparation from cultivated mycelia of Matsutake. As there is no information on composition or concentration/fractionation of the preparation, it is not possible to conclude very much from these studies in relation to consumption of the Matsutake mushroom.

Recommendation

Matsutake is red-listed in Denmark and Norway as near threatened.

Should only be marketed in the Nordic countries, if it is from countries, where it is not red-listed.

Listing 1

Database search information

SciFinder by February 2012. Keywords: *Tricholoma*. PubMed by December 2012. Keywords: *Tricholoma*.

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Tricholoma nauseosum (A. Blytt) Kytöv.

See *Tricholoma matsutake*.

Tricholoma nudum (Bull.) P. Kumm.

See *Lepista nuda*.

Tricholoma personatum s. auct. non (Fr.) P. Kumm.

See *Lepista saeva*.

Tricholoma portentosum (Fr.) Quél.

Charbonnier ("The Coalman") (DK: Grå Ridderhat, FI: Viiruvalmuska, IS: No Icelandic name, NO: Gråmusserong, SE: Streckmusseron.

Background and risk assessment

Charbonnier (*Tricholoma portentosum*) is an edible mushroom.

It forms mycorrhiza with Pine (*Pinus*) and Spruce (*Picea*), more rarely with Beech (*Fagus*), Lime (*Tilia*) and Poplar (*Populus*). It is common in boreal, occasional, but locally common in temperate and hemiboreal and in subalpine/subarctic zones of the Nordic countries (Knudsen & Vesterholt, 2012).

There are no intoxications reported after consumption of Charbonnier, and no natural toxicant constituting a risk for humans has been identified in the mushroom.

Recommendation

No special recommendation.

Listing 2

Database search information

SciFinder by January 2012. Keywords: *Tricholoma*. PubMed by December 2012. Keywords: *Tricholoma* or *Clitocybe*.

References

Knudsen H & Vesterholt J (2012): Funga Nordica. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. Nordsvamp: 1–1083.

Tuber aestivum Vittad. (*T. uncinatum* Chat.)

Summer Truffle (DK: Sommer-Trøffel, FI: ("Kesätryffeli"), IS: Sumartryffill, NO: Sommertrøffel, SE: Sommartryffel (Bourgognetryffel)).

Background and risk assessment

Summer Truffle (*Tuber aestivum*)²⁶ is an appreciated edible mushroom.

It may form mycorrhiza with different deciduous trees: e.g. species of Oak (*Quercus*), Hazel (*Corylus*), Beech (*Fagus*), Hornbeam (*Carpinus*), Hop Hornbeam (*Ostrya*), and Lime (*Tilia*). It grows in calcareous drained soils with a pH not lower than 7. It prefers some shading and grows, for example, in clearings of forests. It is a hypogeous mushroom, which implies that its fruiting bodies are to be found underground, where they may be harvested from late September to January. The mushroom is native to large parts of Europe, from Ireland to Russia and from North Africa to the Nordic countries, where it is found rarely in Sweden and

²⁶ According to Wedén (2004) and Wedén *et al.* (2005), *Tuber aestivum* Vittad. and *T. uncinatum* Chat. are synonyms.

Denmark (Hall *et al.*, 2007; Wang & Liu, 2009; Wedén *et al.*, 2009; Wehr-
len *et al.*, 2009).

Summer Truffle is widely and increasingly cultivated. Around 15% of truffle plantations in Italy are based on inoculated Summer Truffle (Bencivenga *et al.*, 2009). Even at the most northern outposts of wild Summer Truffle, such as in Sweden, truffle orchards have been initiated. In Sweden the first fruiting body was harvested in 2005 on the island of Gotland, and since then the production of this truffle species has increased in Sweden. Some Summer Truffle inoculated trees have also been planted in Denmark, Norway and Finland (Wedén *et al.*, 2009). The potential for cultivation of Summer Truffle and its limitations have recently been reviewed by Stobbe *et al.* (2013).

Inoculated truffle trees, e.g. Hazelnut (*Corylus avellana*) or Common Oak (*Quercus robur*) are produced in nurseries and are planted in orchards with environmental conditions similar to the natural habitats for Summer Truffle. Five to ten years after planting of infected seedlings the harvest may begin (Hall *et al.*, 2007; Wedén, 2009). Summer Truffle is commercialised as fresh, frozen, canned or mixed with foods.

There are no intoxications reported after consumption of Summer Truffle. However, these truffles do contain potentially bioactive constituents, in particular among the volatile organic compounds:

Bioactive constituents

Volatile organic compounds, including flavourings: There are many studies identifying and/or quantifying volatile substances in Summer Truffle. In total more than 50 volatile organic compounds have been identified (e.g., Cuellere *et al.*, 2010; Diaz *et al.*, 2002; 2003; 2009; March *et al.*, 2006; Mauriello *et al.*, 2004; Pelusio *et al.*, 1995; Splivallo *et al.*, 2007; Talou *et al.*, 2001; and as reviewed by Wang & Marcone, 2011). The diverging results presented in these publications may be due to genetic factors, degree of maturity and quality of the truffles, origin of the mushroom, storage conditions (time, temperature), host tree and not at least analytical method (Splivallo *et al.*, 2011; Wang & Marcone, 2011). Also post-harvest treatment of Summer Truffle, such as freeze-drying and subsequent rehydration, modify the aroma-profile as estimated by head-space-gas chromatography-mass spectrometry (Palacios *et al.*, 2012). The major volatile constituents contributing to the flavour of the Summer Truffle are identical to, or structurally/biologically related to flavourings identified in other foodstuffs or used as food additives. These flavouring substances have been risk assessed by the European Food Safety Authority (EFSA) and/or by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). They were found to be of no safety con-

cern at estimated levels of intake. However, most of the sulfur-containing volatiles could not be predicted to be metabolized to innocuous products. Therefore animal studies were considered and No Observed Adverse Effect Levels (NOAEL's) were determined in order to establish safety margins for the food use (EFSA 2012a, 2012b; JECFA 2000). Most studies on volatile substances in Summer Truffle only give relative quantities (percentages) of the single volatiles compared to the total amount of volatile constituents. However, Culleré *et al.* (2010) present quantitative data. In this study 22 aroma-active compounds were identified and quantitative data for all but one of them (dimethyl disulfide) were given. The most important aroma compounds were dimethyl sulfide (8 µg/kg), dimethyl disulfide (not estimated), methional (5 µg/kg), 3-methyl-1-butanol (5 µg/kg), 1-hexene-3-one (5 µg/kg) and 3-ethylphenol (8 µg/kg). The compound found in highest amount was 2-phenylethanol, occurring at 71 µg/kg fresh truffle. However, the amount of aroma-supplying compounds is low in this mushroom, up to 100 times less than in *Tuber melanosporum* (Culleré *et al.*, 2010). With an intake of 20 g Summer Truffle in a meal, consumed four times a month, which must be considered a high intake, there will be a very large safety margin for these aroma-giving compounds in relation to the NOAELs established in animal studies, and which were used for evaluation of the above flavouring substances by EFSA (2012a, 2012b) and JECFA (2000).

The structural formula of dimethyl sulfide is shown below:

Dimethyl sulfide

Coumarins: A study on coumarins occurring in different *Tuber* species analysed 24 samples of Summer Truffle. The species contained 0.9–2.3 mg per kg of the simple coumarin, scopoletin (7-hydroxy-6-methoxycoumarin), 34–84 mg per kg of the angular furocoumarin angelicin (isopsoralen) and 9.4–18 mg per kg of the linear furocoumarin bergapten (5-methoxypsoralen). The identities of the three coumarins were confirmed by mass spectrometry and by comparison with authentic samples (Tirillini & Stoppini, 1996).

Scopoletin (and its glycosides) is widely distributed in the plant kingdom and can be found in for example Cassava (*Manihot esculenta*), Coffee (*Coffea* spp.) and Sweet Potato (*Ipomea* spp.) (Gnonlonfin *et al.*, 2012; Thier *et al.*, 1968). The compound has antibiotic (antifungal) ef-

fects (Peterson *et al.*; 2003 Gnonlonfin *et al.*, 2012). It is not known whether it could have toxic effects in humans but any intake from this truffle is likely to result in very low exposure.

Angelicin is an angular furocoumarin found in some food plants. For example, parsnip (*Pastinaca sativa*) may contain levels between 1 and 28 mg per kg fresh weight, and even much higher levels if injured or infected (Baumann *et al.*, 1988; Ostertag *et al.*, 2002; Peroutka *et al.*, 2007). There are no data on oral administration of the compound angelicin to humans and no adequately controlled oral studies supplying angelicin to experimental animals (Søborg *et al.*, 1995). However, it is known that topical application of angelicin followed by UVA irradiation may give rise to skin tumours in mice (Mullen *et al.*, 1984). IARC has considered the potential carcinogenicity of angelicin and concluded that there neither were adequate studies to evaluate the carcinogenicity of angelicin (without UVA irradiation) in experimental animals nor any adequate studies in humans (IARC, 1986b). A worst case exposure scenario based on an intake of 20 g Summer Truffle, containing the highest level of angelicin found in the mushroom (84 mg/kg) would result in an exposure to 1.68 mg angelicin per meal. If, for instance 4 such meals are consumed per month, this would correspond to an average daily intake of less than 4 microgram angelicin per kg body weight per day for a person weighing 60 kg, which would constitute a very limited risk for potential adverse effects in consumers of Summer Truffle.

Bergapten and xanthotoxin (8-methoxypsoralen) are linear furocoumarins occurring in food plants such as citrus fruits, celery/celeriac, parsnip and parsley leaf and root in quantities from about 1 to 60 mg per kg of apparently healthy fresh fruit or vegetable (Peroutka *et al.*, 2007; Schulzova *et al.*, 2012; Søborg *et al.*, 1995).

The International Agency for Research on Cancer (IARC) has concluded that bergapten is genotoxic *in vitro*, and phototoxic and carcinogenic in combination with long-wave ultraviolet (UVA) irradiation. Overall IARC has concluded that there are inadequate data to evaluate the local or systemic carcinogenic effects of bergapten itself in experimental animals, and that evidence for carcinogenicity to humans is inadequate (IARC 1986a; 1987a; 1987b). The average daily intake of furocoumarins (mainly bergapten and xanthotoxin) has been estimated to be 1.3 mg in the USA and 1.2 mg in Great Britain, corresponding to 0.020–0.022 mg furocoumarins per kg body weight for a person weighing 60 kg. These intakes lie 2–3 orders of magnitude lower than the lowest doses reported to be toxic in sub-chronic and chronic animal studies with xanthotoxin (in the absence of light), 25–30 times lower than the therapeutic doses of bergapten or xanthotoxin (0.5–0.6 mg per kg body

weight) when used as drugs, and 10 times lower than the lowest phototoxic doses (0.23 mg/kg body weight). Consumption of foods with the above average levels of furocoumarins was concluded not to represent a significant risk for phototoxic effects and the risk for skin cancer to be insignificant (DFG-Senate Commission for Food Safety 2004/2006). As the average intake of furocoumarins (0.020–0.022 mg per kg body weight) is 10 times lower than the amount required to reach detectable blood levels, it is unlikely that the average dietary intake of furocoumarins (including bergapten) constitute a cancer risk (Søborg *et al.*, 1995). Based on virtually the same data, Dolan *et al.* (2010) more recently concluded that the risks for phototoxic and carcinogenic effects from dietary furocoumarins are low.

Based on the information available, it can be concluded that the highest level of bergapten found in fresh Summer Truffle (18 mg/kg) will result in low exposures. In a worst case scenario (consumption of 20 g mushrooms four times a month) the exposure would be about 1 microgram bergapten per kg body weight per day, which would constitute a very limited risk for potential adverse effects in consumers of Summer Truffle.

The structural formulae of angelicin and bergapten are shown below.

Mutagens: An Italian symposium report indicated that aqueous and, in particular, DMSO-extracts of Summer Truffle induce micronuclei in root tips of field beans exposed to the extract (Lanza *et al.*, 2004). Such effects might indicate a genotoxic activity of compounds in the mushroom. However, as this information has not been confirmed in an extended study, it should be evaluated with care. Fratianni *et al.* (2007) tested aqueous and ethanolic extracts of fresh Summer Truffle for mutagenic activity in the Ames test, using the *Salmonella typhimurium* strains TA98 and TA100 with and without metabolic activation. The truffle extracts did not induce mutations in this test system under the conditions used.

Recommendation

Red-listed in Denmark as near threatened.

Should only be marketed in the Nordic countries if it comes from cultivation or from countries where it is not red-listed.

Listing 1

Database search information

SciFinder by January 2012. Keywords: *Tuber aestivum*. PubMed by December 2012. Keywords: *Tuber aestivum* or *Tuber uncinatum*.

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Tuber indicum Cooke & Massee (*T. sinense* X.L. Mao)

("Chinese Truffle") (DK: Kinesisk Trøffel, FI: ("Kiinalainen musta tryffeli"), IS: No Icelandic name, NO: No Norwegian name, SE: (Kinesisk tryffel)).

Background and risk assessment

*Tuber indicum*²⁷ is an edible mushroom. The taste is rather bland, but as *T. indicum* is at least one order of magnitude cheaper than *T. melanosporum* and *T. magnatum* there is a large export to Europe and the USA. In France, for instance, the import of Chinese truffles may exceed the official production of *T. melanosporum* (Hall *et al.*, 2007). Except for the different flavours of the two species of fresh truffles, it is almost impossible to distinguish *T. indicum* from *T. melanosporum*. This requires microscopic examination or modern DNA techniques (e.g. specific PCR) (Hall *et al.*, 2007).

T. indicum forms mycorrhiza with many species of trees, both deciduous and coniferous, e.g. *Fagaceae* (*Quercus* spp.), and *Pinaceae* (*Pinus* spp.). It is often found in forests with Pine trees or Pine mixed with deciduous trees, usually in calcareous soils with clay and high pH at altitudes up to 3,000 m above sea level. It grows in the northwest Himala-

²⁷ *Tuber indicum* is now considered the preferred name instead of *T. sinensis* (Chen *et al.*, 2011)

yas in India, but is especially abundant in the Chinese provinces Yunnan and Sichuan. Alone from Yunnan more than 300 tons are harvested each year. More recently China has also initiated cultivation of *T. indicum* and other *Tuber* species (Bonito *et al.*, 2011; Chen *et al.*, 2010; Garcia-Montero *et al.*, 2010; Hall *et al.*, 2007; Wang & Liu, 2009). *T. indicum* has a rather long shelf-life (Bellesia *et al.*, 2002) and is mainly sold as fresh in the Nordic countries. It is also sold as preserved, sometimes fraudulently as *T. melanosporum* (Garcia-Montero *et al.*, 2010; Hall *et al.*, 2007).

There are no intoxications reported after consumption of *T. indicum*. However, *T. indicum* truffles contain potentially bioactive constituents, in particular among the volatile organic compounds:

Bioactive constituents

Volatile organic compounds, including flavourings: There are some studies identifying and/or quantifying volatile substances in *T. indicum*. In total more than 30 volatile organic compounds have been identified in the mushroom (e.g. Bellesia *et al.*, 2002; Cuelleré *et al.*, 2013; Palacios *et al.*, 2012; Splivallo *et al.*, 2007; Talou *et al.*, 2001). The diverging results presented in these publications may be due to genetic factors, degree of maturity and quality of the truffles, origin of the mushroom, storage conditions (time, temperature), host trees and not at least analytical method (Splivallo *et al.*, 2011; Wang & Marcone, 2011). Whereas Bellesia *et al.* (2002) were unable to detect any sulfur-compounds in *T. indicum* using headspace-gaschromatography-mass spectrometry, such compounds were identified by three other research teams. Palacios *et al.* (2012) found dimethyl disulfide and dimethyl trisulfide, Culleré *et al.* (2013) identified dimethyl sulfide and dimethyl disulfide, and Splivallo *et al.* (2007) methylthiopropional (methional). All four research teams conclude that the content of volatile organic compounds is lower in *T. indicum* than in other edible truffles, e.g., *T. melanosporum*.

The major volatile constituents contributing to the flavour of *T. indicum* are identical to or structurally/biologically related to flavourings identified in other foodstuffs or used as food additives. These flavouring substances have been risk assessed by the European Food Safety Authority (EFSA) and/or by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). They were found to be of no safety concern at estimated levels of intake. However, most of the sulfur-containing volatiles could not be predicted to be metabolized to innocuous products. Therefore animal studies were considered and No Observed Adverse Effect Levels (NOAEL's) were determined in order to establish safety margins for the food use (EFSA 2012a, 2012b; JECFA 2000).

Splivallo *et al.* (2007) quantified the major volatile organic compounds produced by *T. indicum* and accounted for in total 82 mg per kg truffle: 3-methyl-1-butanol (33 mg/kg), 1-hexanol (17 mg/kg), 1-octen-3-ol (16 mg/kg), 2-phenylethanol (8 mg/kg), trans-2-octenal (7 mg/kg) and 3-octanone (2 mg/kg), but only traces of the sulfur compounds. In their evaluation of the aroma-active constituents of different *Tuber* species, Liu *et al.* (2012) made use of several different approaches and characterized 12 aroma-active volatile constituents in *T. indicum*/*T. sinensis*. It was concluded that 3-(methylthio)propanal (methional), 3-methylbutanal and 1-octen-3-ol were the key contributors to the aroma of *T. indicum*. The content of these three flavouring substances were 40/40, 340/100 and 40/36 mg per kg fresh truffle, respectively (Liu *et al.*, 2012). With an intake of 20 g *T. indicum*, consumed four times a month, there will be a very large safety margin for these aroma-giving compounds in relation to the NOAELs established in animal studies, and which were used for evaluation of the above flavouring substances by EFSA (2012a, 2012b) and JECFA (2000).

Steroids: A volatile steroidal compound, 5 α -androst-16-en-3 α -ol has been isolated from *T. indicum* fermentation broth (Wang *et al.*, 2008). It is not known, whether also the fruit bodies of *T. indicum*, like the fruit bodies of *T. melanosporum* and of *T. magnatum* produce 5 α -androst-16-en-3 α -ol (Claus *et al.*, 1981).

Recommendation

No special recommendation.

Listing 1

Database search information

SciFinder by January 2012. Keywords: *Tuber indicum* or *Tuber sinensis*. PubMed by December 2012. Keywords: *Tuber indicum* or *Tuber sinensis*.

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Tuber magnatum Picco

White Truffle ("Alba Truffle") (DK: Piemonteser-Trøffel ("Hvid Trøffel", "Alba-Trøffel"), FI: ("Valkotryffeli"), IS: ("Tryffill"), NO: Kongetrøffel, SE: Albatryffel ("Piemontetryffel")).

Background and risk assessment

White Truffle (*Tuber magnatum*) is an excellent edible mushroom, highly prized for its culinary properties. Due to its delicious taste and low world production (estimated 50–200 tons per year), it is one of the world's most expensive foods (Hall *et al.*, 1998; 2005; 2007).

It may form mycorrhiza with different deciduous tree species: Italian Alder (*Alnus cordata*), Hazelnut (*Corylus avellana*), Hop Hornbeam (*Ostrya carpinifolia*), White Poplar (*Populus alba*), European Aspen (*Populus tremula*), and species of Oak (*Quercus* spp.), Willow (*Salix* spp.), Lime (*Tilia* spp.) and others (Hall *et al.*, 1998; 2007). It grows in shaded forests with little sunlight, in calcareous, porous, soft and moist soil with a pH of 7.5 or higher. It is a hypogeous mushroom, which means the fruiting bodies are to be found underground, where they may be harvested from October to December. It is native to Italy but is also found in Croatia and sporadically in France, Serbia, Slovenia, Hungary and Switzerland (Bragato *et al.*, 2004; Hall *et al.*, 2007; Wang & Marcone, 2011).

The occurrence of White Truffle in nature is declining, probably due to deforestation, reforestation with non-host trees, soil disturbance, ill adaption of the host trees and the mycorrhizal mushroom to new climate conditions, or a combination of these (Hall *et al.*, 2004; Bencivenga *et al.*, 2009).

Cultivation of White Truffle is difficult: There are very few orchards, and only a few percentages of cultivated truffles are White Truffle. The low production may be due to less successful inoculation in the nurseries, difficulties with establishing suitable grounds, and the long time required before truffles are produced, which is 15 to 20 years after planting of the inoculated seedlings of host trees. However, seedlings inoculated with White Truffle are planted to some extent in habitats of the wild-growing White Truffle (Mello *et al.*, 2006; Bencivenga *et al.*, 2009; Hall *et al.*, 2004).

White Truffle is commercialised mainly as fresh, but to some extent also as frozen, canned or mixed with foods.

There are no intoxications reported after consumption of White Truffle. However, White Truffle contains potentially bioactive constituents, in particular among the volatile organic compounds:

Bioactive constituents

Volatile organic compounds, including flavourings: There are numerous studies identifying and/or quantifying volatile substances in White Truffle. In total more than 100 volatile organic compounds have been detected (e.g., Aprea *et al.*, 2007; Bellesia *et al.*, 1996; Gioacchini *et al.*, 2008; Mauriello *et al.*, 2004; Pelusio *et al.*, 1995; Pennazza *et al.*, 2013; Talou *et al.*, 2001; and as reviewed by Wang & Marcone, 2011). The diverging results presented in these publications may be due to genetic factors, degree of maturity and quality of the truffles, origin of the mushroom, storage conditions (time, temperature), and not least analytical methods (Splivallo *et al.*, 2011; Wang & Marcone, 2011). Storage of the mushroom for 8 days in sealed jars at 4 °C changed the flavour profile with formation of alcohols and acids, indicating ester hydrolysis and some decrease of important sulfur-containing flavourings such as bis-(thiomethyl)methane and dimethyl sulfide (Piloni *et al.*, 2005). Furthermore, yeasts habituating the fruiting bodies of White Truffle can produce volatile organic compounds, e.g. 3-methylbutanol, dimethylsulfide and dimethyldisulfide, also known from the truffle flavour. Such yeast flavouring substances may possibly contribute to the final truffle aroma (Buzzini *et al.*, 2005).

The major volatile flavouring substances identified in White Truffle are identical with or structurally/biologically related to flavourings

identified in other foodstuffs or used as food additives. These flavouring substances have been risk assessed by the European Food Safety Authority (EFSA) and/or by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). They were found to be of no safety concern at estimated levels of intake. However, most of the sulfur-containing volatiles could not be predicted to be metabolized to innocuous products. Therefore, animal studies were considered and No Observed Adverse Effect Levels (NOAEL's) were determined in order to establish safety margins for the food use (EFSA 2012a, 2012b; JECFA 2000). Most studies on volatile substances in White Truffle only give relative quantities (percentages) of the single volatiles compared to all volatile constituents. However, Bellesia *et al.* (1996) do give some quantitative data. These investigators report that the content of bis(thiomethyl)methane, the characteristic flavouring substance in White Truffle, is 59–89 mg/kg, whereas the level of dimethyldisulfide is 0.3–4.6 mg/kg and the level of dimethyltrisulfide 0.2–5.2 mg/kg fresh truffle. With an intake of 20 g White Truffle in a meal, which must be considered large, consumed four times a month, there will be a very large safety margin for these three aroma-giving compounds in relation to the NOAELs established in animal studies, and which were used for evaluation of the above flavouring substances by EFSA (2012a, 2012b) and JECFA (2000).

The structural formula of bis(thiomethyl)methane is shown below.

bis(Thiomethyl)methane

Steroids: A volatile steroidal compound, 5 α -androst-16-en-3 α -ol has been isolated from the fruiting bodies of White Truffle in amounts of 59–62 μ g per kg fresh mushroom. The steroid has a pronounced musk-like scent. It is the major pheromone of the boar, where it has been detected in testes and saliva (Katkov & Gower, 1970; Prelog & Ruzicka, 1944; Prelog *et al.*, 1944). 5 α -Androst-16-en-3 α -ol also occurs in humans, in testes, sweat and urine, primarily in males (Brooksbank & Haslewood, 1961; Gower *et al.*, 1994; Kwan *et al.*, 1997) but it seems to be devoid of androgenic activity (Claus *et al.*, 1981). It is estimated to be excreted with human urine (as glucuronide) at a rate of about 1 mg per day by adult males and at a slower rate by women (Brooksbank & Haslewood, 1961; Brooksbank *et al.*, 1974). Therefore the potential biological effects of 5 α -androst-16-en-3 α -ol after consumption of a meal containing White Truffle will be negligible

in relation to the natural production in humans. For example, 20 g of the truffle would contain about 1 µg of the steroid.

Coumarins: In a study on the occurrence of coumarins in various *Tuber* species, Tirillini and Stoppini (1996) did not find detectable levels of coumarins in White Truffle. This was in contrast to other *Tuber* species studied.

Recommendation

No special recommendation.

Listing 1

Database search information

SciFinder by January 2012. Keywords: *Tuber magnatum*. PubMed by December 2012. Keywords: *Tuber magnatum*.

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Tuber melanosporum Vittad.

(Black Truffle, “Black Winter Truffle”) (DK: Perigord-Trøffel (“Sort Trøffel”), FI: (“Mustatryffeli”), IS: “tryffill”, NO: Perigordtrøffel, SE: Perigordtryffel).

Background and risk assessment

Tuber melanosporum is an excellent edible mushroom, highly prized for its culinary properties. Due to its delicious taste and low world production (estimated to 150 tons per year), it is one of the world’s most expensive foods (Hall *et al.*, 2007).

It may form mycorrhiza with different deciduous trees: species of Oak (*Quercus* spp.), Hazel (*Corylus* spp.), Lime (*Tilia* spp.), Hornbeam (*Carpinus* spp.) and Hop-hornbeam (*Ostrya* spp.). It grows in bright forests on sunny flat slopes or plateaus with calcareous, well aerated and drained soils with a pH optimum of 7.9. It is a hypogeous mushroom, which means the fruiting bodies are to be found underground, where they may be harvested from late November to early March. It is native to France, Spain and Italy but also, to parts of the Balkans and Portugal (Wang & Marcone, 2011; Hall *et al.*, 2007; Mello *et al.*, 2006).

The natural occurrence of *T. melanosporum* is declining, probably due to deforestation, reforestation with non-host trees or too dense planting, soil

disturbance or ill adaption of the host trees and the mycorrhizal mushroom to new climate conditions (Hall *et al.*, 2004; Bencivenga *et al.*, 2009).

To-day most *T. melanosporum* truffles on the market are cultivated ones. Of the truffles marketed in France around 80% originate from truffle orchards. Spain and Italy are other major producers of cultivated *T. melanosporum*. More recently *T. melanosporum* has been successfully cultivated also in many plantations in New Zealand, Australia and the USA, and other countries have initiated cultivation, e.g. China. Inoculated truffle trees, e.g. Hazelnut (*Corylus avellana*) or Downy Oak (*Quercus pubescens*) are produced in nurseries and are planted in orchards with environmental conditions similar to the natural habitats for *T. melanosporum*. Five to ten years after planting of infected seedlings the harvest may begin (Bencivenga *et al.*, 2009; Hall *et al.*, 2007; Mello *et al.*, 2006; Wang & Liu, 2009). In order to improve the yield of the precious fruiting bodies, research on identifying the soil and management of the plantations and not least on improving the process of mycorrhiza and fruit body formation is prioritized. Conditions for research have been facilitated by the recently published sequence of the *T. melanosporum* genome (Martin *et al.*, 2010; Kües & Martin, 2011).

T. melanosporum truffles are commercialised as fresh, frozen, canned or mixed with foods, e.g. edible oil, cheese, salt and meat products.

There are no intoxications reported after consumption of *T. melanosporum*. However, *T. melanosporum* truffles do contain potentially bioactive constituents, in particular among the volatile organic compounds:

Bioactive constituents

Volatile organic compounds, including flavourings: There are numerous studies identifying and/or quantifying volatile substances in *T. melanosporum* and totally more than 130 volatile organic compounds have been detected (e.g., Bellesia *et al.*, 1988; Buzzini *et al.*, 2005; Cuellere *et al.*, 2010; Diaz *et al.*, 2003; March *et al.*, 2006; Jansen *et al.*, 2009; Mauriello *et al.*, 2004; Pelusio *et al.*, 1995; Splivallo *et al.*, 2007; Talou *et al.*, 2001; Vernin *et al.*, 2005; and as reviewed by Wang & Marcone, 2011). The diverging results may be due to genetic factors, degree of maturity and quality of the truffles, origin of the mushroom, storage conditions (time, temperature), host trees and not at least analytical method (Splivallo *et al.*, 2011; Wang & Marcone, 2011). Freeze-drying and subsequent rehydration of *T. melanosporum* did apparently not affect the aroma-profile when estimated by headspace-gas chromatography-mass spectrometry (Palacio *et al.*, 2012). However, freezing the mushrooms at -20 or -80 °C significantly changed the aroma of the thawed mushrooms both when estimated by physical-chemical methods and by a sensory panel (Cullere

et al., 2013). Furthermore yeasts habituating the fruiting bodies of *T. melanosporum* can produce volatile organic compounds, e.g. 3-methylbutanol, dimethyl sulfide and dimethyl sulfide, also known from the truffle flavour. Such yeast flavouring substances may possibly contribute to the final truffle aroma (Buzzini *et al.*, 2005).

The structural formula of dimethyl sulfide is shown below.

dimethyl sulfide

The major volatile flavouring substances identified in *T. melanosporum* are identical with or structurally/biologically related to flavourings identified in other foodstuffs or used as food additives. These flavouring substances have been risk assessed by the European Food Safety Authority (EFSA) and/or by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). They were found to be of no safety concern at estimated levels of intake. However, most of the sulfur-containing volatiles could not be predicted to be metabolized to innocuous products. Therefore animal studies were considered and No Observed Adverse Effect Levels (NOAEL's) were determined in order to establish safety margins for the food use (EFSA 2012a, 2012b; JECFA 2000). Most studies on volatile substances in *T. melanosporum* only give relative quantities (percentages) of the single volatiles compared to all volatile constituents. However, a few investigators present quantitative data (Bellesia *et al.*, 1998; Cuellere *et al.*, 2010; Ney & Freitag, 1980; Splivallo *et al.*, 2007). The most recent of these four publications show that the typical *T. melanosporum* flavour is composed of at least 17 different aroma-giving substances (Cuellere *et al.*, 2010). The most important of these 17 substances for the flavour were reported to be 2,3-butanedione (0.70 mg/kg of fruit bodies), dimethyl disulfide (not quantified), ethyl butyrate (0.05 mg/kg), dimethyl sulfide (0.19 mg/kg), 3-methyl-1-butanol (0.43 mg/kg) and 3-ethyl-5-methylphenol (18 mg/kg). In another study, Ney & Freitag (1980) identified dimethyl sulfide at a level of 8 mg/kg. With an intake of 20 g *T. melanosporum* in a meal, consumed four times a month, which must be considered a high intake, there will be a very large safety margin for these aroma-giving compounds in relation to the NOAELs established in animal studies, and which were used for evaluation of the above flavouring substances by EFSA (2012a, 2012b) and JECFA (2000).

Steroids: A volatile steroidal compound, 5 α -androst-16-en-3 α -ol has been isolated from the fruiting bodies of *T. melanosporum* in amounts of 40–60 μ g per kg fresh mushroom. The steroid can also be formed in fermentation broth of the truffle (Wang *et al.* 2008). The steroid has a pronounced musk-like scent. It is the major pheromone of the boar, where it has been detected in testes and saliva (Katkov & Gower, 1970; Kwan *et al.*, 1997; Prelog & Ruzicka, 1944; Prelog *et al.*, 1944;). 5 α -Androst-16-en-3 α -ol also occurs in humans, in testes, sweat and urine, primarily in males (Brooksbank & Haslewood, 1961; Gower *et al.*, 1994), but it seems to be devoid of androgenic activity (Claus *et al.*, 1981). It is estimated to be excreted with human urine (as glucuronide) at a rate of about 1 mg per day by adult males and at a slower rate by women (Brooksbank & Haslewood, 1961; Brooksbank *et al.*, 1974). Therefore the potential biological effects of 5 α -androst-16-en-3 α -ol after consumption of a meal containing *T. melanosporum* will be negligible in relation to the natural production in humans. For example, 20 g of the truffle would contain about 1 μ g of the steroid.

Coumarins: In a study on coumarins in different *Tuber* species 24 samples of *Tuber melanosporum* were analysed. The species contained 0.0–0.8 mg per kg of the simple coumarin scopoletin (7-hydroxy-6-methoxycoumarin), 0–1.3 mg per kg of the angular furocoumarin angelicin (isopsoralen) and 11–21 mg per kg of the linear furocoumarin bergapten (5-methoxypsoralen). The identities of the three coumarins were confirmed by mass spectrometry and by comparison with authentic samples (Tirillini & Stoppini, 1996).

Scopoletin (and its glycosides) is widely distributed in the plant kingdom and can be found in for example Cassava (*Manihot esculenta*), Coffee (*Coffea* spp.) and Sweet Potato (*Ipomea* spp.) (Gnonlonfin *et al.*, 2012; Thier *et al.*, 1968). The compound has antibiotic (antifungal) effects (Peterson *et al.*; 2003 Gnonlonfin *et al.*, 2012). It is not known whether it could have toxic effects in humans, but any intake from this truffle is likely to result in very low exposure.

Angelicin is an angular furocoumarin found in some food plants. For example, parsnip (*Pastinaca sativa*) may contain levels between 1 and 28 mg per kg fresh weight, and even much higher levels if injured or infected (Baumann *et al.*, 1988; Ostertag *et al.*, 2002; Peroutka *et al.*, 2007). There are no data on oral administration of the compound angelicin to humans and no adequately controlled oral studies supplying angelicin to experimental animals (Søborg *et al.*, 1995). However, it is known that topical application of angelicin followed by UVA irradiation may give rise to skin tumours in mice (Mullen *et al.*, 1984). IARC has

considered the potential carcinogenicity of angelicin and concluded that there neither were adequate studies to evaluate the carcinogenicity of angelicin (without UVA irradiation) in experimental animals nor any adequate studies in humans (IARC, 1986b). A worst case exposure scenario based on an intake of 20 g *T. melanosporum*, containing the highest level of angelicin found in the mushroom (1.3 mg/kg) would result in an exposure to 0.026 mg angelicin per meal. If, for instance 4 such meals are consumed per month, this would correspond to an average daily intake of less than 0.06 microgram angelicin per kg body weight per day for a person weighing 60 kg. This would constitute a very limited risk for potential adverse effects in consumers of *Tuber melanosporum*.

Bergapten and xanthotoxin (8-methoxypsoralen) are linear furocoumarins occurring in food plants such as citrus fruits, celery/celeriac, parsnip and parsley leaf and root in quantities from about 1 to 60 mg per kg of apparently healthy fresh fruit or vegetable (Peroutka *et al.*, 2007; Schulzova *et al.*, 2012; Søbørg *et al.*, 1995). The International Agency for Research on Cancer (IARC) has concluded that bergapten is genotoxic *in vitro*, and phototoxic and carcinogenic in combination with long-wave ultraviolet (UVA) irradiation. Overall IARC has concluded that there are inadequate data to evaluate the local or systemic carcinogenic effects of bergapten itself in experimental animals, and that evidence for carcinogenicity to humans is inadequate (IARC 1986a; 1987a; 1987b). The average daily intake of furocoumarins (mainly bergapten and xanthotoxin) has been estimated to be 1.3 mg in the USA and 1.2 mg in Great Britain, corresponding to 0.020–0.022 mg furocoumarins per kg body weight for a person weighing 60 kg. These intakes lie 2–3 orders of magnitude lower than the lowest doses reported to be toxic in sub-chronic and chronic animal studies with xanthotoxin (in the absence of light), 25–30 times lower than the therapeutic doses of bergapten or xanthotoxin (0.5–0.6 mg per kg body weight) for bergapten and xanthotoxin when used as drugs, and 10 times lower than the lowest phototoxic doses (0.23 mg/kg body weight). Consumption of foods with the above average levels of furocoumarins was concluded not to represent a significant risk for phototoxic effects and the risk for skin cancer to be insignificant (DFG-Senate Commission for Food Safety 2004/2006). As the average intake of furocoumarins (0.020–0.022 mg per kg body weight) is 10 times lower than the amount required to reach detectable blood levels, it is unlikely that the average dietary intake of furocoumarins (including bergapten) constitute a cancer risk (Søbørg *et al.*, 1995). Based on virtually the same data, Dolan *et al.* (2010) more recently con-

cluded that the risk for phototoxic and carcinogenic effects from dietary furocoumarins is low.

Based on the information available, it can be concluded that the highest level of bergapten found in fresh *T. melanosporum* (21 mg/kg) will result in low exposures. In a worst case scenario (consumption of 20 g mushrooms four times a month) the exposure would be about 1 microgram bergapten per kg body weight per day, which would constitute a very limited risk for potential adverse effects in consumers of *T. melanosporum*.

The structural formulae of angelicin and bergapten are shown below.

Recommendation

No special recommendation.

Listing 1

Database search information

SciFinder by January 2012. Keywords: *Tuber melanosporum*. PubMed by December 2012. Keywords: *Tuber melanosporum*.

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Tuber sinense X.L. Mao

See *Tuber indicum*.

Tuber uncinatum Chat.

See *Tuber aestivum*.

Volvaria volvacea (Bull.) P. Kumm.

See *Volvariella volvacea*.

Volvariella volvacea (Bull.) Singer (*Volvaria volvacea* (Bull.) P. Kumm.)

(Paddy Straw Mushroom, Straw Mushroom) (DK: Spiselig Posesvamp, FI: Viljelytuppisieni, IS: Matsokka, NO: Grå sliresopp, SE: Halmslidskivling).

Background and risk assessment

Paddy Straw Mushroom (*Volvariella volvacea*) is an edible mushroom.

It is a saprotrophic mushroom, growing on grass leaves, e.g., from rice in Japan, Southeast Asia and East Africa (Hongo & Izawa, 2006; Pegler, 1977).

There are no intoxications reported after consumption of Paddy Straw Mushroom, and no natural toxicant constituting a risk for humans has been identified in the mushroom. However, several bioactive constituents with potentially biological effects in humans have been identified in the mushroom.

Bioactive constituents

Ergothioneine: Paddy Straw Mushroom contains relatively high amounts of ergothioneine, 537 mg per kg dried mushroom (mean of three samples) (Lo *et al.*, 2012). Ergothioneine has in numerous *in vitro* and in a few *in vivo* studies been shown to have antioxidative and cytoprotective properties against a wide range of cellular stressors (Song *et al.*, 2010).

However, the physiological role of ergothioneine is not well understood. For further data on ergothioneine, see *Lepista nuda*.

Statins: Lovastatin has been isolated in relatively small amounts from Paddy Straw Mushroom, 59 mg per kg dried fruit bodies (Lo *et al.*, 2012). For further data on lovastatin, see *Pleurotus ostreatus*.

Ribosome inactivating proteins: Ribosome inactivating proteins are enzymes that depurinate rRNA's. This depurination inactivates the ribosomes, thus inhibiting protein synthesis (Stirpe & Battelli, 2006; Nielsen & Boston, 2001). Yao *et al.* (1998) have isolated a ribosome inactivating protein, designated volvarin, with a molecular mass of 29 kDa from the fruit bodies. Volvarin acts as an N-glycosidase that depurinates rRNA and inhibits protein synthesis, which has been demonstrated *in vitro* in a rabbit reticulocyte lysate system. The protein also exerted deoxyribonuclease activity on DNA *in vitro*, as well as abortifacient effect when injected into pregnant ICR mice. It is not known whether volvarin is stable during processing of Paddy Straw Mushrooms.

Cytolytic proteins: Paddy Straw Mushroom contains a cytolytic protein, volvatoxin A. Volvatoxin A lyses human erythrocytes, causes ventricular systolic arrest on isolated toad hearts, and results in significant electrocardiographical changes in cats injected with the compound (Lin *et al.*, 1973). Studies by Fassold *et al.* (1976) have shown that volvatoxin A inhibits calcium accumulation in the mitochondria of the heart muscle. Volvatoxin A consists of volvatoxin A₁ of 50 kDa and volvatoxin A₂ of 24 kDa in the ratio 1:3 (Lin *et al.*, 1973). Although both proteins are required for activity, Lin *et al.* (1973) demonstrated that the hemolytic (an probably cytotoxic, cardiotoxic and neurotoxic) activity is carried by volvatoxin A₂. Volvatoxin A₁ seems not to possess any significant biological activity. More recently a mechanism for the disruption of cell membranes have been proposed, based on the formation of membrane pores (Weng *et al.*, 2004; Wu *et al.*, 2006). It was proposed that volvatoxin A₂ contains two functional domains. One is responsible for oligomerization of volvatoxin A₂ proteins and the other for membrane binding and insertion.

Importantly, volvatoxin A is not heat-stable, and its biological activities are completely destroyed by heating at 100 °C for 20 minutes (Lin *et al.*, 1973).

Lectins: Banerjee *et al.* (1982) demonstrated that extracts of mycelia from submerged cultures of Paddy Straw Mushroom had hemagglutinating activity on several types of erythrocytes (sheep, guinea pig, rat, mouse and goat) but not human, the effect being strongest on sheep erythrocytes. Subsequently, a lectin with hemagglutinating activity was isolated and characterized from fruit bodies of Paddy Straw Mushroom

(Lin & Chou, 1984; She *et al.*, 1998). In the study by Lin & Chou (1984) it was shown that volvatoxin has moderately inhibitory effect on tumour growth in mice inoculated with sarcoma 180 cells. Whereas Lin & Chou (1984) reported that it had a molecular weight of 26 kDa and consisted of two non-identical subunits, She *et al.* (1998) reported a homodimeric protein with a molecular weight of 32 kDa. The latter investigator showed that the hemagglutinating activity was inhibited by thyroglobulin but not by simple carbohydrates. The lectin has been shown to have strong immunomodulatory activity as demonstrated by for example mitogenic activity (She *et al.*, 1998; Ho *et al.*, 2004). The lectin was quite stable at elevated temperatures, and 75% percent of the activity remained after incubation at 90°C for 30 minutes (Lin & Chou, 1984).

Recommendation

No special recommendation.

Listing 1

Database search information

SciFinder by January 2012. Keywords: *Volvariella* or *Volvaria*. PubMed by March 2012. Keywords: *Volvariella* or *Volvaria*.

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Xerocomus badius (Fr.) E.-J. Gilbert
(*Boletus badius* (Fr.) Fr.)

Bay Bolete (DK: Brunstokket Rørhat, FI: Ruskotatti, IS: No Icelandic name, NO: Svartbrun rørsopp, SE: Brunsopp).

Background and risk assessment

Bay Bolete (*Xerocomus badius*)²⁸ is regarded as a good edible mushroom, which is mainly sold fresh.

It forms mycorrhiza mainly with coniferous, but also with deciduous trees. Bay Bolete is common in the temperate/hemiboreal and southern boreal zones and occasional in middle-boreal area of the Nordic countries (Knudsen & Vesterholt, 2012).

No intoxications have been reported after consumption of Bay Bolete, and no inherent toxicants have been identified in the mushroom.

One unusual amino acid derivative, N-ethyl- γ -glutamine has been isolated from Bay Bolete (Casimir *et al.*, 1960). This compound has been

²⁸ Index Fungorum/Species Fungorum (2012) uses *Boletus badius* as current name but Knudsen & Vesterholt (2012), ArtDatabanken (2011) and Danish Mycological Society (2012) use *Xerocomus badius* as preferred name.

studied for its anticipated beneficial effects in *in vitro* and rodent studies, e.g. as referred to by Li *et al.* (2008). Whether the occurrence of N-ethyl- γ -glutamine in Bay Bolete may lead to any adverse effects in humans is unknown.

Muszynska *et al.* (2009) isolated small amounts of the indole compounds tryptophan, 5-hydroxytryptophan, tryptamine and of the two metabolites of tryptophan: kynurenic acid and kynurenine sulphate. High intakes of tryptophan can damage the nervous system and contribute to induction of cancer (Stone *et al.*, 2003).

Recommendation

No special recommendation.

Listing 2

Database search information

SciFinder by January 2012. Keywords: *Xerocomus*, *Boletus*.

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4. Pictures and statements on poisonous mushrooms referred to in guidance list 3

Introduction

In guidance list 3 on “Wild edible mushrooms, which may easily be mistaken for poisonous look-alikes and therefore are not regarded as suitable for commercial marketing,” references are made to specific toxic mushrooms. These toxic mushrooms are not evaluated in this report, but a picture of each of these species is found below together with a short statement on their toxicity.

It has to be stressed, that one picture of a mushroom species will never cover all variations in the appearance of the mushroom in nature. The natural appearance may differ significantly and thorough knowledge of identification is an essential part of the safe trade and use of mushrooms as food.

The poisonous mushrooms are listed alphabetically according to their preferred scientific names.

List of toxic look-alikes referred to:

- *Amanita pantherina* (Panthercap).
- *Amanita phalloides* (Deathcap).
- *Amanita virosa* (Destroying Angel).
- *Boletus legaliae* (No English name).
- *Boletus satanas* (Devil's Bolete).
- *Chlorophyllum brunneum* (No English name).
- *Clitocybe rivulosa* (Fool's Funnel).
- *Cortinarius rubellus* (Deadly Webcap).
- *Galerina marginata* (Funeral Bell).
- *Hypholoma fasciculare* (Sulphur Tuft).

Amanita pantherina (DC.) Krombh.

Panthercap (DK: Panter-Fluesvamp, FI: Pantterikärpässieni, IS: No Icelandic name, NO: Panterfluesopp, SE: Panterflugsvamp).

Very poisonous

Contains the neurotoxic isoxazoles, ibotenic acid and muscimol, which are the primary toxicants in the mushroom giving rise to neurotoxic effects with dizziness, ataxi, euphoria, anxiety, blurred vision and possibly tremor and convulsions.

Amanita phalloides (Vaill. ex Fr.) Link

Deathcap (DK: Grøn Fluesvamp, FI: Kavalakärpässieni, IS: Vomuserkur (Grænserkur), NO: Grønn Fluesopp, SE: Lömsk flugsvamp).

Deadly poisonous

Contains three groups of toxic cyclic peptides, of which the bicyclic octapeptides, amanitins, are the primary toxic constituents. The amanitins are cytotoxic compounds which primarily may give rise to very severe gastrointestinal and hepatotoxic effects, often with fatal outcome.

Amanita virosa (Fr.) Bertill.

Destroying Angel (DK: Snehvid Fluesvamp, FI: Valkokärpässieni, IS: No Icelandic name, NO: Hvit fluesopp, SE: Vit flugsvamp).

Deadly poisonous

Contains three groups of toxic cyclic peptides, of which the bicyclic octapeptides, amanitins, are the primary toxic constituents. The amanitins are cytotoxic compounds which primarily may give rise to very severe gastrointestinal and hepatotoxic effects, often with fatal outcome.

Boletus legaliae Pilát

No English name (DK: Djævle-Rørhat, FI: No Finnish name, NO: No Norwegian name, SE: Falsk djävulssopp).

Poisonous

Gives rise to gastrointestinal adverse effects. Toxicant(s) unknown.

Boletus satanas Lenz

Devil's Bolete (DK: Satans Rørhat, FI: No Finnish name, NO: No Norwegian name, SE: Djävulssopp).

Poisonous

Contains bolesatine, a hemagglutinating glycoprotein (lectin), which probably is the toxicant giving rise to the severe gastrointestinal adverse effects, often seen after consumption of Devil's Bolete.

Chlorophyllum brunneum (Farl. & Burt)
Vellinga (*Macrolepiota bohemica* (Wichanský)
Kriegslt. & Pázmány, *Macrolepiota brunneum*
(Farl. & Burt) Wasser)

No English name (DK: Giftig Rabarberhat, FI: No Finnish name.
IS: No Icelandic name, NO: Lumsk parasolsopp, SE: No Swedish name).

Poisonus

May give rise to gastrointestinal adverse effects. Toxicant(s) unknown.

Clitocybe rivulosa (Pers.) P. Kumm.
(*Clitocybe dealbata* (Sowerby) P. Kumm. s. auct.)

Fool's Funnel (DK: Eng-Tragthat, (Bleg Tragthat), FI: Myrkkymalikka,
IS: Mótrektla, NO: Lumsk traktsopp, SE: Gifttrattskivling).

Very poisonous

Contains the neurotoxic quaternary ammonium compound L-(+)- muscarine which gives rise to gastrointestinal and autonomic effects. Severe poisonings including excessive respiratory secretion, bronchospasms and possibly cardiovascular collapse.

Cortinarius rubellus Cooke (*C. speciosissimus* Kühner & Romagn.)

Deadly Webcap (DK: Puklet Gift Slørhat, FI: Suippumyrkkyseitikki, NO: Spiss giftslørhat, SE: Toppig giftspindling).

Deadly poisonous

Contains orellanine, a neurotoxic bipyridine compound, and other related compounds which after a very long latency period of 1–3 weeks may give rise to renal damage or even irreversible failure, sometimes with fatal outcome.

Galerina marginata (Batsch.) Kühner
(*Galerina autumnalis* (Peck) A.H. Sm. & Singer)

Funeral Bell (DK: Randbæltet Hjelmhat, FI: Myrkkynäöpikkä, IS: Viðarkveif, NO: Flatklokkehat, SE: Gifthätting).

Deadly poisonous

Contains the toxic cyclic octapeptides, amanitins, which like for Deathcap primarily may give rise to very severe gastrointestinal and hepatotoxic effects, sometimes with fatal outcome.

Hypholoma fasciculare (Huds.) P. Kumm

Sulphur Tuft (DK: Knippe-Svovlhat, FI: Kitkerälahokka, IS: Brennis-teinsnollur, NO: Besk svovelsopp, SE: Svavelgul slöjskivling).

Poisonous

May give rise to gastrointestinal adverse effects. Toxicants unknown.

Mushrooms traded as food. Vol II sec. 2

Mushrooms recognised as edible have been collected and cultivated for many years. In the Nordic countries, the interest for eating mushrooms has increased.

In order to ensure that Nordic consumers will be supplied with safe and well characterised, edible mushrooms on the market, this publication aims at providing tools for the in-house control of actors producing and trading mushroom products.

The report is divided into two volumes:

- a. Volume I: "Mushrooms traded as food - Nordic questionnaire and guidance list for edible mushrooms suitable for commercial marketing
- b. Volume II: Background information, with general information in section 1 and in section 2, risk assessments of more than 100 mushroom species

All mushrooms on the lists have been risk assessed regarding their safe use as food, in particular focusing on their potential content of bioactive constituents.

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