Published in : Food Additives & Contaminants (2009), vol. 9, pp. 1265-1272
Status : Postprint (Author's version)

Formaldehyde in cultivated mushrooms: a negligible risk for the consumer

W. Claeys[a], C. Vleminckx[b], A. Dubois[a], A. Huyghebaert[c], M. Höfte[c], P. Daenens[d] and B. Schiffers[e]

[a]*Federal Agency for the Safety of the Food Chain, Brussels, Belgium;*
[b]*Scientific Institute of Public Health, Brussels, Belgium;*
[c]*Ghent University, Ghent, Belgium;*
[d]*Catholic University Leuven, Leuven, Belgium;*
[e]*Gembloux Agricultural University, Gembloux, Belgium*

Abstract

Following the detection of formaldehyde in cultivated mushrooms, an evaluation was carried out to assess whether its presence in food poses a risk to public health. Formaldehyde, a carcinogenic chemical, has a broad range of industrial applications and, hence, exposure to formaldehyde is ubiquitous through diverse consumer goods, food, the air, etc. The observed levels of formaldehyde in mushrooms are lower than the levels reported for vegetables, fruit, meat, fish and dairy products. On the basis of available data, a rough estimate of the dietary exposure to formaldehyde was performed. The exposure through the consumption of cultivated mushrooms (approximately 0.19 µg kg^{-1} body weight day^{-1} on average, consumers only) appeared to be small compared with the total dietary intake of formaldehyde (approximately 99.0 µg kg^{-1} body weight day^{-1}, total population). Based on comparison with toxicological safety limits for chronic exposure and given that formaldehyde is carcinogenic only through inhalation and not by ingestion, it can be concluded that the dietary exposure to formaldehyde is not a cause for concern.

Keywords : high-performance liquid chromatography (HPLC) ; natural toxicants ; pesticide residues ; mushrooms

INTRODUCTION

Formaldehyde (CH_2O, CAS 50-0-0) is a colourless, flammable gas that is commercially available as a 35-40% aqueous solution (formalin), as formol (a mixture of formaldehyde, formic acid and methanol in water) or as the precursor hexamethylenetetramine (a complex of formaldehyde with ammonium). In the solid form formaldehyde is available as the trimer trioxane (1,3,5-trioxane or s-trioxane) or as the polymer paraformaldehyde ($(CH_2O)_n$ with $n \geq 8$) (International Programme on Chemical Safety (IPCS) 1989, 2002).

Formaldehyde is classified by the International Agency on Cancer (IARC) into Group 1, as being carcinogenic to humans (IARC 2006). Within the European Union formaldehyde is currently classified as a category 3 carcinogen with the risk phrase 'R40, limited evidence of a carcinogenic effect' (Directive 2001/59/EC; European Commission 2001), but discussions are currently taking place to change this classification to category 1 - 'R49, may cause cancer by inhalation'. Most studies regarding the toxicity of formaldehyde relate to the inhalation of formaldehyde, which is probably the most important route of exposure. The available data on the effects of ingestion or of skin contact with formaldehyde are limited.

Since formaldehyde is water soluble, highly reactive with biological macromolecules (formaldehyde induces DNA-protein and protein-protein cross-links), and rapidly metabolized, the effects of exposure are mainly observed in those tissues or organs which come into first contact with formaldehyde, namely the respiratory and gastrointestinal tract, oral and gastrointestinal mucosa included (erosion, ulceration, inflammation and hyperplasia of stomach and forestomach were observed in rats) (IPCS 1989, 2002; Schulte et al. 2006; Bundesinstitut fur Risikobewertung (BfR) 2006; IARC 2006). There is no evidence that formaldehyde is carcinogenic by the oral route (European Food Safety Authority (EFSA) 2006). Formaldehyde causes toxicity to the nasal epithelium of rats and mice upon inhalation and induces above certain concentrations dose-related increases in nasal tumours. Epidemiological data have shown that formaldehyde is carcinogenic in human by the inhalation route (nasopharyngeal cancers and sinonasal cancers). While a genotoxic mode of action (MOA) can never be ruled out for a compound that is clearly genotoxic, at least in vitro and locally in vivo, the MOA would be based on the induction of sustained cytoxicity and cell proliferation at the site of contact upon long-term exposure (McGregor et al. 2006).

Formaldehyde is produced industrially for a large number of applications such as the production of resins that act as adhesives and binders for wood products, pulp, paper, glass wool and rock wool, and the production of some plastics, coatings, paints and varnishes, industrial chemicals and textile finishing. It is also used in packaging, cosmetics and as a disinfectant and preservative. For example, formaldehyde is currently allowed as a preservative under the form of hexamethylene tetramine (E 239) in Provolone cheese at a residual concentration of 25 mg kg^{-1} expressed as formaldehyde (Directive 95/2/EC; European Commission 1995). For materials and articles made of plastic that come into contact with food, a specific migration limit (SML) of 15 mg kg^{-1} is set for formaldehyde (Directive 2002/72/EC; European Commission 2002).

In the context of its monitoring programme, the Belgian Federal Agency for the Safety of the Food Chain (FASFC) analysed formaldehyde in cultivated mushrooms. In Belgium, the use of formaldehyde as a disinfectant in the cultivation of mushrooms is not allowed. However, there are biocides admitted that contain formaldehyde and the regulation provides the possibility of exemptions for mushroom substrates and fertilizers (Royal Decree of 7 January 1998 on the trade in fertilizers, soil improvers and growing substrates). Currently, there are no European or Belgian standards for formaldehyde in mushrooms. The main objective of this paper is to evaluate if the presence of formaldehyde in cultivated mushrooms poses a risk to consumers and if regular control of formaldehyde in cultivated mushrooms is necessary. To place the potential risk into perspective, the total dietary exposure to formaldehyde is considered as well.

MATERIALS AND METHODS

Samples

A first batch of mushrooms (Agaricus, Pleurotus) was sampled in the context of the monitoring programme of the FASFC. Mainly mushrooms of Belgian origin were sampled, but also imported mushrooms were considered (Table 1). Next, an inquiry was performed at corresponding Belgian production sites, where a second mushroom sample (ready for harvesting), a sample of the compost bed and a sample of the casing soil were taken. Samples were stored frozen until analysis.

Table 1. *Formaldehyde concentration (mg kg^{-1}) measured in cultivated mushrooms, compost bed and casing soil.*

Mushroom sample 1	Mushroom sample 2	Compost bed	Casing soil	Remarks
0.35	0.40	0.23	0.02	Belgian origin, conventional farming
0.27	0.08	0.10	<LOQ	Belgian origin, conventional farming
0.31	0.13	0.36	0.02	Belgian origin, conventional farming
0.30	0.21	0.12	0.05	Belgian origin, conventional farming
0.21	0.53	0.49	0.06	Belgian origin, organic farming
	0.56	0.49	0.06	Belgian origin, organic farming
	0.16	0.28		Belgian origin, organic farming
0.39	0.38	0.44	0.04	Belgian origin, conventional farming
0.42				Belgian origin, conventional farming
0.53	0.18	0.05	0.02	Belgian origin, conventional farming
0.21				Belgian origin, conventional farming
	0.18	0.27	0.03	Belgian origin, conventional farming
	0.30	0.20	0.03	Belgian origin, conventional farming
0.16	0.07	0.34		Belgian origin, conventional farming; 5.1 mg kg^{-1} formaldehyde was measured in supplementary feeding
0.18	0.09	0.30		Belgian origin, conventional farming
0.65				Belgian origin, conventional farming
0.24				Dutch origin, organic farming
0.28				Polish origin, conventional farming
0.34				Polish origin, conventional farming
0.45				Polish origin, conventional farming

Note: LOQ, limit of quantification (0.02).

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Analysis of formaldehyde

Formaldehyde levels in mushrooms and substrates were analysed after extraction with acetonitrile and derivatization with dinitrophenylhydrazine (DNPH) by high-performance liquid chromatography (HPLC) with ultra violet-diode array detection (HPLC-UV-DAD; Varian, Middelburg, The Netherlands), according to the method described by Tomkins et al. (1989). For extraction, fresh mushroom (100 g) was mixed with 1-acetonitrile (100 ml) and placed for 30min in an ultrasonic bath and for 30min on an orbital shaker. After filtration, 5 ml DNPH derivatization reagent were added (i.e. 300 mg DNPH dissolved in 50 ml acetonitrile with 0.5ml H_3PO_4 (85%)). The mixture was further diluted to 200 ml with acetonitrile and placed for 3h in a thermostatic oven at 40°C. After filtration on a membrane filter (0.45 µm), 20 µl of sample were injected on a Luna C18 column (250 x 4.6 mm, 5µm; Phenomenex, Utrecht, The Netherlands). Mobile phase A was acetonitrile, mobile phase B was a 0.02 M NaH_2PO_4 solution in acetonitrile at pH 4 (50:50 v/v). Gradient elution started at 0% A, rising after 12min linearly to 60% A over 2 min, then held at 60% A for a further 6 min before returning to the initial conditions. The total injection cycle time was 24 min and the flow rate was 1.0 ml min^{-1}. The presence of formaldehyde was confirmed by comparison of the samples' retention time (approximately 9.6 min) and characteristic spectrum with a standard sample (formaldehyde solution > 36.5%; Sigma-Aldrich, Bornem, Belgium) at 353 nm. The limit of quantification (LOQ) for 100 g of sample was determined as 0.02mgkg^{-1}.

Consumption data

Consumption data were obtained from the Belgian Food Consumption Survey (BFCS) performed in 2004 and coordinated by the Scientific Institute of Public Health (Devriese et al. 2005). The survey involved 3214 participants older than 15 years, which were interviewed twice about their consumption during the last 24h (non-consecutive 24-h recall). Participants were selected by a multistage procedure from the National Register and the fieldwork was spread over 1 year to anticipate seasonal effects. The usual food intake was estimated by the Nusser method using C-side software podd 1996).

RESULTS AND DISCUSSION

Occurrence of formaldehyde

Formaldehyde is ubiquitous. Possible anthropogenic sources of formaldehyde are combustion (vehicular exhaust, waste, cigarettes, etc.), industrial applications (resins, paints, etc.), and consumer goods (cosmetics, pesticides, contact materials, etc.).

In the atmosphere, formaldehyde is both directly emitted and formed as a result of photochemical oxidation of reactive organic gases. The concentration of formaldehyde in the air is generally below 0.001 mg m^{-3} in rural areas and below 0.020 mg m^{-3} in urban areas (IARC 2006). The indoor air may contain higher levels of formaldehyde compared with the outdoor air due to evaporation from furniture, paints and construction materials. Reported levels for indoor air range between 0.02 and 0.06mg m^{-3} (IARC 2006). The formaldehyde level due to occupational exposure (e.g. during the varnishing of furniture and wooden floors, in the finishing of textiles, in the garment industry, in the treatment of fur and in certain jobs within manufactured board mills and foundries, etc.) is on average 2 mg kg^{-1} (Heck and Casanova 2004; IARC 2006).

In rainwater, formaldehyde concentrations of 0.1-0.2 mg l^{-1} are measured (IPCS 1989). In drinking water, formaldehyde is mainly formed by natural oxidation of humic substances during the ozonation and chlorination of the water or is released into the water from plastic plumbing. Water treated with ozone likely contains less than 50 µg l^{-1} formaldehyde (World Health Organization (WHO) 2005; Owen et al. 1990).

Formaldehyde is also naturally present in the majority of living organisms. As an intermediary metabolic product formaldehyde is essential for the biosynthesis of certain amino acids. In vivo most formaldehyde is probably (reversibly) bound to macro-molecules (IPCS 2002). The content of endogenously metabolically formed formaldehyde can range between 3 and 12 ng g^{-1} tissue (Owen et al. 1990). The endogenous concentration of formaldehyde measured in blood is 2-3 mg l^{-1} (IARC 2006). A similar concentration was found in the blood of rats and monkeys (Heck et al. 1985; Casanova et al. 1988) and a two to four times higher concentration was observed in the liver and the nasal mucosa of rats (Heck et al. 1982). Additionally, formaldehyde is a natural component of a variety of foodstuffs. Monitoring the formaldehyde level of food, however, has generally been performed sporadically and source-directed and only few data are available to characterize the range and distribution of formaldehyde concentrations in food (IPCS 2002). In the context of the monitoring programme, the FASFC analysed the formaldehyde level of cultivated mushroom samples. The concentration ranged between 0.08 and 0.65 mg kg^{-1} (n = 29) (Table 1). It seems that the formaldehyde concentration of organically cultivated mushrooms (on average 0.34 ± 0.19 mg kg^{-1} (n = 5)), is similar to the concentration measured in conventionally cultivated mushrooms (on average 0.29 ± 0.15 mg kg^{-1} (n = 24)). (The production of organic food within the European Union, as well as the importation of organic food from outside the European Union is controlled by European Union Regulation 2092/91; European Commission 1991.) No correlation is observed between mushrooms and compost bed or casing soil with respect to the formaldehyde concentration. Moreover, there seems to be no transfer of formaldehyde between substrate treated with formaldehyde and mushrooms (internal communication, data not given). At the production site no indications were found of unauthorized treatment of substrates (compost bed and casing soil) with formaldehyde. Data given in Table 1 thus concern endogenously formed formaldehyde.

In the literature, studies considering the presence of formaldehyde in mushrooms are scarce. Existing data concern Shiitake mushrooms, which have a relatively high formaldehyde level. In a study of the British Food Safety Agency (FSA) formaldehyde concentrations in the order of 100-300 mg kg^{-1} were observed in both fresh and partially dried Shiitake mushrooms due to natural production. These formaldehyde concentrations refer, however, to a combination of free and 'bound' formaldehyde (i.e. formaldehyde derived from the breakdown of larger molecules by thermal degradation, by acidic and enzymatic hydrolyses, e.g. during analysis). After 6min of cooking, the formaldehyde level was significantly decreased. Preservation for 10 days had no effect on the formaldehyde content of the mushrooms (Mason et al. 2004). In an opinion of the French Agence Française de Sécurité Sanitaire des Aliments (AFSSA), it was concluded that a maximum formaldehyde level of 63 mg kg^{-1} in fresh Shiitake mushrooms can be considered safe for the consumer (AFSSA 2001). Formaldehyde levels observed in the present study are well below these values.

For comparison, literature data regarding the formaldehyde concentration of different foodstuffs are presented in Table 2. Formaldehyde levels range between 3 and 60 mg kg^{-1} for vegetables and fruits, between <1 and 34 mg kg^{-1} for fish and meat (for crustaceans between 1 and 98 mg kg^{-1}), and around 1 mg kg^{-1} for milk and milk products. For alcoholic beverages, soft drinks and coffee, formaldehyde levels between 0.02 and 16.3 mg l^{-1} are reported. It should be noted, however, that literature data are scarce and that data presented in Table 2 could be biased (e.g. because they are based on a limited number of samples). Most of the levels reported in Table 2 are due to the natural occurrence of formaldehyde, but it can not be excluded that some values are a result of processing (e.g. fumigation, the use of formaldehyde-containing food additives, migration from melamine-, phenol- and urea-formaldehyde plastics, the addition to feed to improve handling characteristics or as a preservative, etc.). Moreover, the analytical method used to quantify formaldehyde may affect the formaldehyde level measured since there are many potential precursors that can form formaldehyde under certain extraction and derivatization conditions used during analysis (Mason et al. 2004).

Table 2. *Formaldehyde level of different foodstuffs reported in literature.*

Food	**Concentration (mg kg^{-1})**	**Remarks**	**Reference**
Fruit and vegetables			
Apple	17.3 (38.7) 6.3-22.3	Colorimetric determination using chromotropic acid (Schiff's reagent)	**a** **b**
Banana	16.3		**b**
Pear	60 (38.7)	Colorimetric determination using chromotropic acid (Schiff's reagent)	**a**
Cauliflower	4.7 (5.3) 26.9	Colorimetric determination using chromotropic acid (Schiff's reagent)	**a** **b**
Carrot	6.7 (10)	Colorimetric determination using chromotropic acid (Schiff's reagent)	**a**
Onion	13.3 (26.3)	Colorimetric determination using chromotropic acid (Schiff's reagent)	**a**
Spinach	3.3 (7.3)	Colorimetric determination using chromotropic acid (Schiff's reagent)	**a**
Tomato	5.7 (7.3)	Colorimetric determination using chromotropic acid (Schiff's reagent)	**a**

Radish	3.7 (4.4)	Colorimetric determination using chromotropic acid (Schiff's reagent)	**a**
Shiitake mushroom	100-406/6-54.4 100-320	Colorimetric determination using chromotropic acid and dimedone/ammonium acetate derivative measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS)	**b** **c**
Meat products			
Beef	0.094 ± 0.044	France	**d**
	0.079 ± 0.051 and 0.161 ± 0.071	European Member States	**d**
Poultry	5.7	Colorimetric analysis	**a**
	2.3-5.7		**d**
Pork	20	Colorimetric determination using chromotropic acid	**a**
Sheep	8		**a**
Beef, pork, sheep and chicken	2.5-20		**b**
Fish products			**a**
Freshwater fish	8.8	Fumigated, colorimetric analysis	**a**
Sea fish	20	Fumigated, colorimetric analysis	**a**
Cod	20	Frozen	**a**
	4.6-34		**b**
Crustaceans	1-60	Mediterranean	**a**
	3-98	Ocean	**a**
Shrimp	1	Live, high-performance liquid chromatography (HPLC)	**a**
Milk and diary products			
Milk	0.041 ± 0.045	France	**d**
Goats milk	1		**a**
Cows milk	Up to 3.3	Colorimetric analysis	**a**
Fresh milk	0.013-0.057	(mean = 0.027 mgl^{-1})	**e**
Treated milk	0.075-0.255	(mean = 0.164mgl^{-1})	**e**
Cheese	Up to 3.3	Colorimetric analysis	**a**
Dairy products	0.02		**d**
Beverages			
Alcoholic beverages	0.04-1.7	Fluorescence detection after on-line condensation with 4-amino-3-penten-2-one (Japan)	**e**
	0.02-3.8	Spectrofluorimetric determination (Brazil)	**e**
Soft drinks	7.4-8.7	Four samples of canned and bottled cola (Canada); HPLC-MS of DNPH-derivative	**f**
	< 2 (LOD)[1]	Five samples of canned soft drink (UK); HPLC of DNPH derivative	**8**
Beer	0.1-1.5	Nine samples of canned and bottled beer (Canada); HPLC-MS of DNPH-derivative	**f**
	< 1 (LOD)[1]	Five samples of canned beer (UK); HPLC of DNPH derivative	**g**
Coffee	3.4-4.5	Higher levels of formaldehyde were found in instant coffees than in brewed coffees,	**h**
Instant coffee	10-16.3	perhapsbecause formaldehyde escapes from coffee during brewing (USA)	**h**

Notes: [a]International Programme on Chemical Safety (IPCS) (1989); [b]Yau (2008); [c]Mason et al. (2004); [d]Agence Française de Sécurité Sanitaire des Aliments (AFSSA) (2004); International Programme on Chemical Safety (IPCS) (2002); [f]Lawrence and Iyengar (1983); [g]Food Safety Authority (FSA) (1998); [h]Hayashi et al. (1986); [i]Limit of detection (LOD).

Formaldehyde intake

Table 3 presents the intake of formaldehyde through the consumption of cultivated mushrooms based on monitoring data of the FASFC (Table 1) and consumption data of the BFCS (Devriese et al. 2005). The intake was calculated by a deterministic approach multiplying a fixed value for consumption with a fixed value for the formaldehyde level (such as the mean or P97.5), and concerns only consumers of mushrooms and not the whole population. One of the main criticisms of the deterministic approach is its inherent conservatism. Nevertheless, due to its simplicity and its worldwide use and acceptance, this point estimate approach may be used as a screening tool.

***Table 3**. Intake of formaldehyde by the consumption of mushrooms.*

	Formaldehyde (mg kg^{-1})[a]	Consumption (g day^{-1})[b]	Intake[c]	
			mg day^{-1}	µg kg^{-1} body weight day^{-1}
Mean	0.288	49	0.01	0.19
P50	0.275	34	0.01	0.12
P95	0.547	160	0.09	1.15
P97.5	0.585	200	0.12	1.54
P99	0.624	233	0.15	1.91
P100	0.650	465	0.30	3.98

Notes: [a]Twenty-nine samples (see Table 1). [b]Usual consumption of the Belgian population ('consumers only') - 460 consumption days (Devriese et al. 2005). tabulated deterministically; the body weight was assumed to be 76 kg.

On average, the formaldehyde intake due to the consumption of cultivated mushrooms was 0.2 µg kg^{-1} body weight day^{-1}. In the (upper) worst-case scenario, the intake could amount to 4.0 µg kg^{-1} body weight day^{-1}. In the FSA study cited above, the formaldehyde intake due to consumption of Shiitake mushrooms was estimated to be 0.15-0.16 mg kg^{-1} body weight. It was concluded that such an intake level poses probably no appreciable risk to public health (Mason et al. 2004). The intake determined in the FSA study is one to two orders of magnitude higher than estimated in the present study. Not only different types of mushrooms were considered in both studies, but also different analytical methods were applied. As mentioned above, the analytical conditions used can affect the formaldehyde level measured due to the breakdown by thermal degradation, acidic and enzymatic hydrolyses of larger molecules. In the FSA study, formaldehyde levels were determined after extraction by acid hydrolysis and steam distillation using a spectrophotometric method and LC-MS. Formaldehyde formation and potential routes for its generation in mushrooms by steam distillation under acidic conditions have been described in the literature (Tyihak et al. 1996; and Yamazaki et al. 1980, cited by Mason et al. 2004).

Additionally, the total dietary exposure of formaldehyde was estimated deterministically for the whole population (Table 4). Based on the formaldehyde data available in the literature, an average formaldehyde intake of approximately 7.5 mg day^{-1} (0.10 mg kg^{-1} body weight day^{-1}) was obtained (beverages not included). This is a rough estimate of the actual intake, for one because it was assumed that each person consumes each day average amounts of fruit, vegetables, meat or fish, milk and dairy products. Also, formaldehyde levels used for calculations were taken arbitrarily as the average between the minimum and maximum concentration found in literature and are as such biased (see above). In addition, the effect of, for example, cooking before consumption on the formaldehyde content, which can lower the formaldehyde content of the food by evaporation, was not accounted for. Moreover, the proportion of formaldehyde in foods that is bioavailable and the amount that is in a bound and unavailable form, is unknown (IPCS 1989, 2002; Health Canada 2001).

A similar exercise was performed by Owen et al. (1990), who calculated an annual dietary formaldehyde intake of about 4000 mg year^{-1}, equivalent to approximately 11 mgday^{-1}. The intake through drinking water was calculated to be on average less than 40µg day^{-1}. The rough estimate given by the International Programme on Chemical Safety (IPCS 1989) for formaldehyde exposure through food is of the same range, namely between 1.5 and 14 mg day^{-1} for an average adult.

It is clear that the intake through the consumption of mushrooms is negligible compared with the intake of formaldehyde via the consumption of different food products (only 0.2%).

When considering the 'total' exposure to formaldehyde, other routes of exposure should also be considered such as inhalation and skin absorption by dermal contact (e.g. by use of cosmetics). The latter route of exposure can be considered negligible (except in certain workplaces), whereas the first route is probably the most important one. Probabilistic simulations of Health Canada indicate that through the air one in every two people would be exposed to 24-h average formaldehyde concentrations of ≥ 20-24 µg l^{1} (24-29 µgm^{-3}) and that one in 20 people (i.e. the 95th percentile) would be exposed to 24-h average formaldehyde concentrations in air ≥ 67-78 µg l^{1} (80-94 µg m^{-3}) (Health Canada 2001). The German Bundesinstitut fur Rizikobewertung (BfR) established in a recent toxico-logical evaluation a tolerable air concentration of 0.1 mg l^{-1} formaldehyde, which is in line with the maximum allowable concentration (MAC) of 0.3mg l^{-1} determined to protect workers in the workplace (BfR 2006).

Table 4. *Formaldehyde intake by the consumption of different foodstuffs.*

	Formaldehyde (mg kg^{-1})[a]	Average consumption (g day^{-1})[b]	Intake[c] mg day^{-1}	µg kg^{-1} body weight day
Vegetables[d]	15.1	138.3	2.1	27.48
Fruit	33.2	118.2	3.9	51.56
Meat	10.0	120.7	1.2	15.92
Fish and seafood	49.5	23.9	1.2	15.57
Milk and dairy beverages	1.7	90.6	0.2	1.97
Cheese	1.7	30.2	0.1	0.66
Yoghurt and pudding	1.7	63.1	0.1	1.38

Notes: [a]Concentration = average of the minimum and the maximum concentration reported (see Table 2); it was assumed that the preparation of food (e.g. cooking) did not reduce the formaldehyde concentration. [b]Usual average consumption of the Belgian population (Devriese et al. 2005). [c]Cabulated deterministically; the body weight was assumed to be 76 kg. [d]Shiitake mushrooms excluded.

Risk characterization

The WHO guideline for drinking water sets a maximum limit of 900µg formaldehyde l^{-1} water (WHO 2006). The limit was determined on the basis of a tolerable daily intake (TDI) of 0.15 mg kg^{-1} body weight day^{-1} and assuming that drinking water accounts for 20% of the intake. This TDI was established on the basis of a no observed adverse effect level (NOAEL) of 15 mg kg^{-1} body weight day from a 2-year study in rats where irritations of the stomach and papillary hyperplasia were observed at a formaldehyde dose of 82 mg kg^{-1} body weight day^{-1}. Based on the same NOAEL, the US Environmental Protection Agency (USEPA) determined a chronic reference dose (RfD) of 0.2 mg kg^{-1} body weight day^{-1} for oral exposure (USEPA 1990). The formaldehyde intake through consumption of mushrooms estimated in this study is 0.10% of the chronic USEPA RfD and 0.13% of the TDI determined by the WHO. As to the total dietary intake, it amounts to more or less 50% and 66% of the USEPA and WHO safety limits, respectively.

The risk associated with the formaldehyde levels measured in food thus appears to be acceptable. Moreover, in an opinion regarding the use of formaldehyde as a preservative in food manufacturing the EFSA AFC Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food concluded on the basis of recent and past toxicological evaluations that there is no evidence indicating that formaldehyde is carcinogenic by the oral route (EFSA 2006).

CONCLUSION

The levels of formaldehyde detected in cultivated mushrooms are generally lower than those reported to occur naturally in vegetables, fruit, meat, fish and dairy products. The consumption of mushrooms results in a formaldehyde intake 1000 times lower than the toxicological safety limits given by the WHO and the USEPA and contributes only little to the total formaldehyde intake. Regular control of formaldehyde in cultivated mushrooms is thus not a priority.

Due to a lack of data, it is difficult to assess precisely the total dietary intake. Nevertheless, the rough estimate presented in this paper is in line with values reported elsewhere and is 66% of the WHO TDI of 0.15 mg kg^{-1} body weight day^{-1}. Since probably not all formaldehyde is bioavailable and since there are no indications that formaldehyde is carcinogenic via the oral route, it can be concluded that the natural occurrence of formaldehyde in food presents no immediate cause for concern.

Acknowledgement

The authors would like to acknowledge the Scientific Committee of the Belgian Federal Agency for the Safety of the Food Chain for their guidance of this study.

References

Agence Française de Sécurité Sanitaire des Aliments (AFSSA). 2001. Avis de l'Agence française de sécuritésanitaire des aliments relatif a un projet d'arrêté relatif a la teneur maximale en formaldehyde que doivent presenter les champignons de l'espèce shiitaké (Lentinus edodes) [internet]. 24 September 2001. Maisons-Alfort, Paris (France): AFSSA; [cited 2008 Oct 6]. Available from: http://www.afssa.fr/Documents/AAAT2001sa0228.pdf/

Agence Française de Sécurité Sanitaire des Aliments (AFSSA). 2004. Evaluation des risques liés à l'utilisation du formaldehyde en alimentation animale [internet], January 2004. Maisons-Alfort, Paris (France): AFSSA; [cited 2008 Oct 6]. Available from: http://www.afssa.fr/ Documents/ALAN-Ra-formaldehyde.pdf/

Bundesinstitut für Risikobewertung (BfR). 2006. Toxicological assessment of formaldehyde. Opinion of BfR No. 023/2006 [internet]. 30 March 2006. Berlin (Germany): Bundesinstitut für Risikobewertung; [cited 2008 Oct 6]. Available from: http://www.bfr.bund.de/cm/ 290/toxicological_assessment_of_formaldehyde.pdf/

Casanova M, Heck H d'A, Everitt J, Harrington Jr W, Popp J. 1988. Formaldehyde concentrations in the blood of rhesus monkeys after inhalation exposure. Food Chem Toxicol. 26:715-716.

Devriese S, De Backer G, De Henauw S, Huybrechts I, Kornitzer K, Leveque A, Moreau M, Van Oyen H. 2005. The Belgian food consumption survey: aims, design and methods. Arch Public Health. 63:1-16.

Dodd K. 1996. A technical guide to C-SIDE. Software for Intake Distribution Estimation. Technical Report Number 96-TR 32, 1-68. Ames (IA): Department of Statistics and Center for Agricultural and Rural Development, Iowa State University.

European Commission. 1991. Council Regulation (EEC) No. 2092/91 of 24 June 1991 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs. Off J L 198:1.

European Commission. 1995. Commission Directive 95/2/ EC of 20 February 1995 on food additives other than colours and sweeteners. Off J. L 61:1.

European Commission. 2001. Commission Directive 2001/ 59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Off J. L 225:1.

European Commission. 2002. Commission Directive 2002/ 72/EC of 6 August 2002 relating to plastic materials and articles intended to come into contact with foodstuffs. Off J. L 220:18.

European Food Safety Authority (EFSA). 2006. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to the use of formaldehyde as a preservative during the manufacture and preparation of food additives (Question N° EFSA Q-2005-032). EFSA J. 415:1-10; [cited 2008 Oct 6]. Available from: http://www.efsa.europa.eu/ EFSA/Scientific_Opinion/afc_op_ej415_formaldehyde_op_ en,2.pdf/

Food Safety Authority (FSA). 1998. MAFF UK - Survey of chemical migration from can coatings into food and beverages - 1, Formaldehyde [internet]. September 1998. London(UK): Food Safety Agency; [cited 2008 Oct 6], Available from: http://archive.food.gov.uk/maff/archive/ food/infsheet/1998/nol57/157form.htm#ref2/

Hayashi T, Reece CA, Shibamoto T. 1986. Gas chromatographic determination of formaldehyde in coffee via thiazolidine derivative. J Assoc Off Anal Chem. 69:101-105.

Health Canada. 2001. Canadian Environmental Protection Act, 1999. Priority substances list assessment report. Formaldehyde [internet]. February 2001. Quebec (Canada): Health Canada; [cited 2008 Oct 6]. Available from: http://www.hc-sc.gc.ca/ewh-semt/alt_formats/hecs-sesc/pdf/pubs/conta-minants/psl2-lsp2/formaldehyde/formal-dehyde-eng.pdf/

Heck H d'A, Casanova M. 2004. The implausibility of leukemia induction by formaldehyde: a critical review of the biological evidence on distant-site toxicity. Regul Toxicol Pharmacol. 40:92-106.

Heck H d'A, Casanova-Schmitz M, Dodd P, Schachter E, Witek T, Tosun T. 1985. Formaldehyde (CH20) concentrations in the blood of humans and Fischer-344 rats exposed to CH20 under controlled conditions. Am Ind Hyg Assoc J. 46:1-3.

Heck H d'A, White E, Casanova-Schmitz M. 1982. Determination of formaldehyde in biological tissues by gas chromatography/mass spectrometry. Biomed Mass Spectrom. 9:347-353.

International Agency for Research on Cancer (IARC). 2006. Formaldehyde, 2-butoxyethanol and l-tert-butox-2-pro-panol. Monographs on the Evaluation of Carcinogenic Risks to Humans 88: 2-9 [internet]. 2006. Paris (France): IARC; [cited 2008 Oct 6]. Available from: http://mono-graphs.iarc.fr/ENG/Monographs/vol88/mono88.pdf/

International Programme on Chemical Safety (IPCS). 1989. International Programme on Chemical Safety, formaldehyde. Environmental Health Criteria [internet]. Geneva (Switzerland): WHO; [cited 2008 Oct 6]. Available from: http://www.inchem.org/documents/ehc/ehc/ehc89.htm/

International Programme on Chemical Safety (IPCS). 2002. International Programme on Chemical Safety, formaldehyde. Concise international chemical assessment document [internet]. 2002. Stuttgart (Germany): WHO; [cited 2008 Oct 6]. Available from: http://www.inchem.org/docu-ments/cicads/cicads/cicad40.htm/

Lawrence J, Iyengar J. 1983. The determination of formaldehyde in beer and soft drinks by HPLC of the 2,4-dinitrophenylhydrazone derivative. Intern J Environ Anal Chem. 15:47-52.

Mason DJ, Sykes MD, Panton SW, Rippon EH. 2004. Determination of naturally-occurring formaldehyde in raw and cooked Shiitaki mushrooms by spectrophotometry and liquid chromatography-mass spectrometry. Food Addit Contam. 21:1071-1082.

McGregor D, Bolt H, Cogliano V, Richter-Reichhelm HB. 2006. Formaldehyde and glutaraldehyde and nasal cytotoxicity: case study within the context of the 2006 IPCS human framework for the analysis of a cancer mode of action for humans. Crit Rev Toxicol. 36:821-835.

Owen BA, Dudney CS, Tan EL, Easterly CE. 1990. Formaldehyde in drinking water: comparative hazard evaluation and an approach to regulation. Regul Toxicol Pharmacol. 11:220-236.

Schulte A, Bernauer U, Madle S, Mielke H, Herbst U, Richter-Reichhelm H-B, Appel K-E, Gundert-Remy U. 2006. Assessment of the carcinogenicity of formaldehyde [CAS No. 50-00-0] [internet]. February 2006. Berlin (Germany): Bundesinstitut fur Risikobewertung (BfR); [cited 2008 Oct 6]. Available from: http://www.bfr. bund.de/cm/238/assessment_of_the_carcinogenicity_of_ formaldehyde. pdf/

Tomkins BA, McMahon JM, Caldwell WM, Wilson DL. 1989. Liquid chromatographic determination of total formaldehyde in drinking water. J Assoc Off Anal Chem. 72:835-839.

Tyihak E, Blunden G, Yang M-H, Crabb T, Sardi E. 1996. Formaldehyde, as its dimedone adduct, from Ascophyllum nodosum. J Appl Phycol. 8:211-215.

US Environmental Protection Agency (USEPA). 1990. Integrated Risk Information System (IRIS) - formaldehyde [internet]. January 1990. Washington (DC): US Environmental Protection Agency, [cited 2008 Oct 6], Available from:http://www.epa.gov/iris/subst/0419.htm/

World Health Organization (WHO). 2005. Formaldehyde in drinking-water. Background document for development of WHO Guidelines for drinking-water quality. Geneva (Switzerland): WHO. (WHO/SDE/WSH/ 05.08/48) [internet], [cited 2008 Oct 6]. Available from: http://www.who.int/water_sanitation_health/dwq/ chemicals/formaldehyde 130605. pdf/

World Health Organization (WHO). 2006. Guidelines for drinking-water quality. 3rd ed. p. 194 [internet]. Geneva (Switzerland): WHO, [cited 2008 Oct 6]. Available from: http://www.who.int/water_sanitation_health/dwq/ gdwq3/en/

Yamazaki H, Ogasawara Y, Sakai C, Yoshiki M, Makino K, Kishi T, Kakiuchi Y. 1980. Studies on formaldehyde in Lentinus edodes. J Food Hyg Soc Japan. 21:165-170.

Yau A. 2008. Formaldehyde in food [internet]. April 2008. Hong Kong (China): Centre forFood Safety (CFS) - The Government of the Hong Kong Special Administrative Region, [cited 2008 Oct 6]. Available from: http:// www.cfs.gov.hk/english/multimedia/multimedia_pub/multimedia_pub_fsf_06_01 .html/