SCIENTIFIC OPINION

Scientific Opinion on the abiotic risks for public and animal health of glycerine as co-product from the biodiesel production from Category 1 animal by-products (ABP) and vegetable oils[1]

EFSA Panel on Contaminants in the Food Chain (CONTAM)[2, 3]

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ABSTRACT

Following a request from the European Commission, the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the abiotic risks for public and animal health of glycerine as co-product from the biodiesel production from Category 1 animal by-products (ABP) and vegetable oils. Crude glycerine is used as an energy-rich feed component in animal diets. Glycerine derived from biodiesel produced from vegetable oils may contain up to 0.5 % methanol and 1 % sodium, used as catalysts in the biodiesel production process. Inclusion rates of such produced glycerine at levels of up to 15 % in the diet of ruminants and up to 10 % in monogastric animals are well-tolerated and exerted no adverse effects on animal health. In contrast, these findings would need to be confirmed for crude glycerine derived from biodiesel production using feedstocks (input materials) other than vegetable oils, as the production process is slightly different. More importantly, no data are available on the levels of possible contaminants of crude glycerine derived from biodiesel production using recycled animal fats or animal by-products classified as category 1 materials due to chemical risk. Therefore, the Panel on Contaminants in the Food Chain recommends the collection of data on the presence of impurities and contaminants in crude glycerine from biodiesel production that is used as animal feed component.

KEY WORDS

biodiesel, feed, risk assessment, crude glycerine, methanol, sodium, ABP-products

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SUMMARY

Following a request from the European Commission, the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the risks for public and animal health of glycerine as a co-product from the biodiesel production from Category 1 animal by-products (ABP) and vegetable oils. The Panel on Contaminants in the Food Chain (CONTAM Panel) noted that the increasing demand for alternative fuels for diesel engines has resulted in a significant increase in biodiesel production. Subsequently, diverse input materials (feedstocks) including recycled animal fats and ABP are used for this purpose. The main co-product of biodiesel production is crude glycerine that is used as an energy-rich feed component in animal diets. Previous assessments of crude glycerine from biodiesel production were confined to the evaluation of biodiesel produced from vegetable oils, intended for human consumption. Data show that inclusion rates of such produced glycerine up to a level of 15 % in the diet of ruminants and up to 10 % in the diet of monogastric animals had no adverse effects on animal health. These findings indicated also that the residual amounts of methanol (up to 0.5 % in the crude glycerine fraction) and sodium (up to 1 % in crude glycerine) used as catalysts in the biodiesel process exerted no adverse effects. These findings are in agreement with the known toxicological data for the individual compounds, but need to be confirmed for glycerine derived from biodiesel using other sources than vegetable oils as feedstock material, as in this case the production process can be different.

The CONTAM Panel noted that no data are available regarding other possible contaminants of crude glycerine derived from biodiesel production. Therefore, the CONTAM Panel recommends the collection of data on the presence of impurities and contaminants in crude glycerine from biodiesel production that is used as animal feed component. This applies in particular to recycled animal fats and Category 1 ABP material, the latter being defined as entire bodies or parts of animals or products of animal origin not intended for human consumption. These products may contain residues of environmental contaminants and other substances and remain of concern to human and animal health unless it is proven that the chemical processes involved in the trans-esterification of the feedstock in the biodiesel production inactivate these abiotic (chemical) contaminants.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

In the Opinion of the Panel on Biological Hazards (BIOHAZ) of the European Food Safety Authority (EFSA) on "Biodiesel process" as a method for safe disposal of Category I Animal by-Products (ABP), adopted on 2 June 2004,[4] the Panel concluded that as the input material has undergone a treatment of 133° C /20 minutes / 3 bar rendering the resulting biodiesel, as well the by-products such as potassium sulphate and glycerine, do not carry a TSE risk.

By letter on 7 April 2008, the Commission requested EFSA to clarify if on the basis of the abovementioned opinion and the opinion of the Scientific Steering committee on six alternative methods for safe disposal of animal by-products, adopted on 10-11 April 2003,[5] if the use of glycerine produced as co-product would pose any risk to public or animal health.

The BIOHAZ Panel discussed this at its meeting on 16-17 April 2008 and agreed that due to the very strict conditions to which the input material is submitted during the biodiesel production process, the use of the resulting glycerine in animal feed is considered to be unlikely to present a microbiological risk provided that, after production, handling practices do not give rise to external contamination. However it was indicated that the possible abiotic risks to public or animal health related to the use of glycerine in feed need to be assessed by the Scientific Panel on Contaminants in the Food Chain (CONTAM).

The letter from EFSA to the European Commission of 26 May 2008 (reference MH/FB/lf out-3026064), indicated that the abiotic issues would be discussed at a future meeting of the CONTAM Panel.

Biodiesel can also be produced from vegetable oils and glycerine is also produced as a by-product of this process. Glycerine obtained as a co-product from the production of biodiesel from vegetable oils contains minimum 80 % of glycerine, maximum 0.5 % of methanol and about 1.2 % of non-glycerine organic material. The Agence Française de Sécurité Sanitaire des Aliments (AFSSA) has issued on 2 May 2007 an opinion on the harmlessness of glycerine for use in animal feed (AFSSA - Saisine n° 2007-SA-0013).[6]

Therefore, the Commission asked EFSA by letter on 11 September 2009 (reference D(2009) FV/ng/420228) to look also in detail to the abiotic risks in glycerine produced as a by-product from the biodiesel production from vegetable oils.

In particular, EFSA is requested to assess the possible abiotic risks for public and animal health of glycerine produced as co-product from the biodiesel production from Category I animal by-products (ABP) and vegetable oils. Related to the biodiesel production process, the possible additional input of chemical contaminants already present in the input material should be evaluated.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Art 29 (1) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority for a scientific opinion on the abiotic risks for public and animal health of glycerine obtained as co-product from the biodiesel production from Category I animal by-products (ABP) and vegetable oils.

[4] http://www.efsa.europa.eu/en/scdocs/doc/23.pdf.
[5] http://ec.europa.eu/food/fs/sc/ssc/out352_en.pdf.
[6] http://www.afssa.fr/Documents/ALAN2007sa0013.pdf.

In particular, the opinion should assess the risks for public and animal health related to the use of certain chemical compounds such as e.g. the use of methanol in the biodiesel production process from Category I animal by-products (ABP) and vegetable oils.

ASSESSMENT

1. Introduction

Biodiesel, as an alternative fuel for diesel engines, is produced from biological sources such as vegetable oils and animal fats. Vegetable oils that are used for biodiesel production include soybeans, sunflower, rapeseed and palm. More recently also non-conventional plant oils such as tobacco, pongamia, jatropha and rubber seeds have been tested for their suitability in biodiesel production (Karmakar et al., 2010). The basic process of biodiesel production is the chemical reaction (trans-esterification) of the triglycerides with an alcohol, usually methanol, that is added during this process (Moser, 2009). This reaction requires the addition of catalysts, often strong bases such as sodium or potassium hydroxide. The reaction causes the breakdown of the triglycerides into methyl esters (biodiesel) and glycerine, the major co-product of biodiesel production. With every litre of biodiesel produced, approximately 79 g of crude glycerine are generated (Thompson and He, 2006).

Major impurities of the crude glycerine fraction are up to 0.5 % methanol (added in excess to drive the reaction), soaps (as a result of side-reactions), and most of the catalyst, particularly sodium salts up to an amount of 1.4 % (Van Gerpen, 2005; Pyle et al., 2008) and 1.2 % organic non-glycerine matter and some water (AFSSA, 2007). Refining of the crude glycerine requires the separation of the soaps (achieved by acidification to split off free fatty acids) and separation of the methanol (achieved by flash evaporation or distillation). These further refining steps can yield glycerine of up to 99.5-99.7 % purity (Van Gerpen, 2005) and these pure forms are suitable for use by the cosmetic and pharmaceutical industries (USP grade). However, refining methods are costly and large amounts of the crude glycerine remain unrefined, particularly when used as a feed supplement (Lammers et al., 2007; Hansen et al., 2009).

The increasing demand for biodiesel has resulted in the use of low quality animal fats and/or recycled fats and oils from domestic and commercial processes as a feedstock (input material) for biodiesel production. Their high fatty acid content, albeit in a different composition to the traditionally used vegetable oils, makes them perfect sources for biodiesel production. The technological process differs only marginally from that used for vegetable oils, in that acidic catalysts are used, including sulphuric acid (H_2SO_4), hydrochloric acid (HCl) and sulphurous acid (H_2SO_3). Their use is determined by the feedstock specifications, especially the content of free fatty acids and water (Aranda et al., 2008). In addition, heterogeneous catalysts are used, including enzymes, titanium-silicates, alkaline-earth, metal compounds, anion exchange resins and guanidine, heterogenized on organic polymers. As with production using vegetable oils, crude glycerine is the major co-product.

Animal fats used for biodiesel production may include also animal by-products (ABP). ABP are defined as entire bodies or parts of animals or products of animal origin, not intended nor fit for human consumption. ABP are categorised in 3 categories of risk, where Category 1 includes ABP of high risk and Category 3 presents low risk ABP. Category 1 material may also comprise condemned carcasses and other products of animals, which are not in compliance with the provisions of Council Directive 96/22/EC[7] (prohibited substances) and Council Directive 96/23/EC.[8]

Therefore, given the high diversity of the feedstock material that may be used in biodiesel production, the European Food Safety Authority (EFSA) was requested to assess the possible risks for public and animal health of glycerine produced as a co-product of the biodiesel production from vegetable oils and animal by-products (ABP), considering that crude glycerine is widely used as a feed component.

[7] OJ L125, 23.5.1996, p. 3-9.
[8] OJ L125, 23.5.1996, p. 10-32.

Previously, the Scientific Panel on Biological Hazards (BIOHAZ) concluded that it is unlikely that microbiological risks are associated with the use of crude glycerine due to the strict handling of the input material during the biodiesel production process. Complementary to this assessment, the Scientific Panel on Contaminants in the Food Chain (CONTAM) is requested to evaluate the possible abiotic risks to public or animal health related to the use of glycerine as feed material.

2. EU legislation related to biodiesel production and glycerine

Biodiesel production

In general, the production of biodiesel is regulated by Regulation (EC) No 92/2005.[9] In Annex IV of this Regulation, biodiesel production is also defined as a treatment of the fat fraction of animal by-products (animal fat) under certain conditions referring to processing methods described in Regulation (EC) No 1774/2002.[10] Regulation (EC) No 1774/2002 lays down rules concerning the means of disposal and uses of animal by-products (ABP). This Regulation divides ABP into 3 categories of risk, where Category 1 includes ABP of high risk material and Category 3 presents ABP with a low risk to animal and humans. This input material (denoted 'feedstock') may comprise products derived from animals treated with substances prohibited under Council Directive 96/22/EC[7] or carcasses or products of animal origin containing residues of environmental contaminants and other substances listed in Council Directive 96/23/EC.[8] In 2007, both above mentioned Regulations were modified by Regulation (EC) No 1576/2007[11] in order to permit additional end uses of Category 1, Category 2 and Category 3 materials and to lay down principles for additional surveillance on initial implementation of the processes for the treatment of Category 1 material.

The process of biodiesel production is defined in Annex IV of Regulation (EC) No 92/2005 as follows: *The processed fat is separated from the protein and insoluble impurities are removed to a level not exceeding 0.15 % in weight, and subsequently submitted to esterification[12] and trans-esterification. However, esterification is not required for Category 3 processed fat. For esterification the pH is reduced to less than 1 by adding sulphuric acid (H_2SO_4; 1.2-2 molar) or an equivalent acid and the mixture is heated to 72 °C for 2 hours during which it is intensely mixed. Trans-esterification shall be carried out by increasing the pH to about 14 with 15 % potassium hydroxide (KOH; 1-3 molar) or with an equivalent base at 35 °C to 50 °C for at least 15 to 30 minutes. Trans-esterification shall be carried out twice under the conditions described above using a new base solution. This process is followed by refinement of the products including vacuum distillation at 150° C, leading to biodiesel and separating the by-products[13] such as glycerine.*

Glycerine

Specific purity criteria for glycerine (E 422) as a food additive are laid down in Commission Directive 2008/84/EC.[14]

Glycerine was also authorised as an additive in feedingstuffs under Directive 70/524/EEC[15] in all species or categories of animals and in all feedingstuffs. No maximum content was set in the

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[9] OJ L19, 21.1.2005, p. 27-33.
[10] OJ L273, 10.10.2002, p. 1-95.
[11] OJ L340, 22.12.2007, p. 89-91.
[12] "*esterification and trans-esterification*" are used in the original text of the Regulation; however, the CONTAM Panel decided to use the correct terms"esterification and trans-esterification" in the opinion.
[13] "*byproducts*" used here for glycerine from the biodiesel production process was left since the original legislative text uses this terminology instead of "co-products".
[14] OJ L 253, 20.9.2008, p.76.
[15] OJ L 270, 14.12.1970, p. 1–17.

consolidated list[16] published in 2004. At present, glycerine is no longer to be considered as a feed additive within the scope of Regulation No 1831/200317, which replaced Directive 70/524/EEC but as a feed material (Commission Regulation (EU) 892/2010).[18] No maximum levels are set for the use of glycerine as a feed material.

Glycerine is included in the list of feed materials of the latest version of the Catalogue of feed materials, which received a favourable opinion of the Standing Committee on the Food Chain and Animal Health on 18 November 2010[19] and which will replace Commission Regulation (EU) No 242/2010 of 19 March 2010 creating the Catalogue of feed materials.[20]

In the catalogue of feed materials, "Glycerine, crude" is described as "Product of biodiesel production (methyl or ethyl esters of fatty acids), obtained by trans-esterification of oils and fats of unspecified vegetable and animal origin with subsequent refining of glycerine (minimum content of glycerol: 99 % of dry matter) Mineral and organic salts might remain in the crude glycerine (maximum content of methanol 0.2 %). Also a product of oleochemical processing of mineral fats and oils, including trans-esterification, hydrolysis or saponification are mentioned in this Catalogue.

The glycerol, potassium and sodium content of crude glycerine and glycerine intended for use as feed material are legally required to be declared.

3. Glycerine

Glycerine (1,2,3-propanetriol), also commonly called glycerine or glycerol, is a tri-alcohol organic compound. Glycerine is present in the form of its esters (glycerides) in all animal and vegetable fats and oils. Glycerine is widely used as a solvent, antifreezer, plasticiser and sweetener, and in the manufacture of cosmetics, liquid soaps, inks, lubricants and dynamite. Several grades of glycerine are marketed including high gravity, yellow distilled, U.S. Pharmacopoeia (USP grade), and chemically pure. USP grade is suitable for use in foods, pharmaceuticals and cosmetics, or for any purpose where the product is designed for human consumption.

Crude glycerine (typically containing 20 % water and residual esterification catalyst) is nowadays mostly obtained as a co-product of saponification and trans-esterification from biodiesel production. Glycerine is also synthesised on a commercial scale from propylene (obtained by cracking petroleum). The conventional epichlorhydrin process for glycerine synthesis is no longer economically viable.

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[16] OJ C50, 25.2.2004, p.19.

[17] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on addtives for use in animal nutrition, OJ L 268, 18.10.2003, p. 29.

[18] Commission Regulation (EU) No 892/2010 of 8Octrober 2010 on the status of certain products with regard to feed additives within the scope of Regulation (EC) No 1831/2003 of the European Parliament and of the Council, OJ L266, 08.10.2010, p.8.

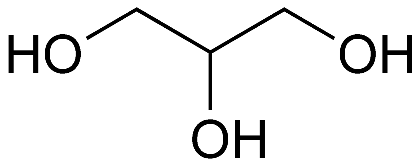
[19] Summary record of the Standing Committee on the Food Chain and Animal Health, section Animal Nutrition, held on 18-19 November in Brussels, agenda item 6, http://ec.europa.eu/food/committees/regulatory/scfcah/animalnutrition/sum_1819112010_en.pdf

[20] OJ L 77, 24.3.2010, p. 17.

Glycerine (synonyms: glycerin, glycerol, 1,2,3-propanetriol) structure and properties

C.A.S. number 56-81-5

Molecular formula: $C_3H_8O_3$



Molecular weight: 92.09 g/mol

Boiling point: 290 °C

Melting point: between 18 and 20 °C

Description: clear, colourless, hygroscopic, syrupy liquid; miscible with water and with ethanol.

In the living organisms, glycerine is a precursor for synthesis of triacylglycerides and of phospholipids in the liver and adipose tissue. When the body uses stored fat as a source of energy, glycerine and fatty acids are released into the bloodstream. The glycerine component can be converted to glucose by the liver and provides energy for cellular metabolism. Glycerine must be converted to the intermediate glyceraldehyde 3-phosphate before it can enter the pathway of glycolysis or gluconeogenesis (depending on physiological conditions).

3.1. Common use of Glycerine

Use of glycerine as a food additive

Specific purity criteria for glycerine (E 422) as a food additive are laid down in Commission Directive 2008/84/EC.[14] Glycerine is used in foods and beverages as a humectant, solvent and sweetener, and may help preserve foods. It is used as a filler in commercially prepared low-fat foods (e.g. cookies), and as a thickening agent in liqueurs. Glycerine is also used to manufacture mono- and di-glycerides for use as emulsifiers, as well as polyglycerine esters that are used in shortenings and margarine. Glycerine-containing hyperhydration solutions are regularly used by athletes (Kavouras et al., 2005).

Use in pharmaceutical products and processes

Glycerine is also used in medical, pharmaceutical and personal care preparations, mainly as a means of providing lubrication and as a humectant. It is found in cough syrups, elixirs, toothpaste, skin care products, shaving cream and hair care products.

Glycerine has anti-freeze properties, disrupting hydrogen bonding when dissolved in water, thus preventing ice crystal formation. The minimum freezing point is at about 60-70 % glycerine in water. Glycerine is therefore a common solvent for enzymatic reagents, biotechnical products, tissue samples and cells used in biomedical research.

Use as a feed material

Currently glycerine is classified as a feed material according to Commission Regulation (EU) 892/2010[21] (see above, section 2).

[21] Commission Regulation (EU) No 892/2010 of 8 October 2010 on the status of certain products with regard to feed additives within the scope of Regulation (EC) No 1831/2003 of the European Parliament and of the Council, OJ L 266, 08.10.2010, p.8.

3.2. Previous assessments on glycerine (from biodiesel production)

3.2.1. Assessment of the BIOHAZ Panel

At its meeting held on 16 and 17 April 2008, the BIOHAZ Panel considered whether the use of glycerine produced as a co-product of the biodiesel process (including the use of diverse feedstocks as defined in Commission Regulation (EU) No 892/2010, such as oils and fats of unspecified vegetable and animal origin with subsequent refining of glycerine) would pose any risk to public or animal health. The BIOHAZ Panel agreed that, due to the rigid chemical procedures to which the input material is submitted during the biodiesel production process, the use of the resulting glycerine in animal feed is considered to be unlikely to present a microbiological risk, provided that after production handling practices do not give rise to external contamination (EFSA, 2008). However, it was indicated that the possible abiotic risks to public or animal health related to the use of glycerine in feed needed to be assessed by the CONTAM Panel.

3.2.2. Opinion of the Agence française de sécurité sanitaire des aliments (AFSSA)

The former AFSSA[22] addressed the use of glycerine for use in animal feeds. This opinion deals specifically (and exclusively) with glycerine obtained as a co-product of the biodiesel production process from vegetable oils intended for human consumption. In addition, this opinion refers to a previous assessment on the use of glycerine as a feed material in pigs (AFSSA, 2007).

This crude glycerine as described in the AFFSA documents contains at least 80 % glycerine and a maximum of 0.5 % methanol, 5 % salts (mainly in the form of sodium chloride), 1.2 % organic non-glycerine substances; the remaining fraction would be water.

AFSSA concluded that the use of this kind of glycerine as a raw material in animal feed presents no risk when the maximal incorporation level does not exceed 10 % of a complementary/complete standardized feed at 88 % dry matter (DM) (AFSSA, 2007). AFFSA addressed also the safety of the residual amounts of methanol, corresponding in the above-mentioned specification to 500 mg/kg feed at 88 % dry matter. It was stated that this methanol concentration could be considered as safe, but emphasised that no evaluation could be presented regarding the safety of this glycerine-methanol mixture when used in feed for marine species.

3.2.3. United States Food and Drug Administration (FDA)

The FDA considers that glycerine is generally recognised as safe (GRAS) in animal feed when used in accordance with good manufacturing or feeding practice (FDA, 2010a, b).

3.2.4. Joint Food and Agriculture Organisation/World Health Organisation (FAO/WHO) Expert Committee on Food Additives (JECFA)

In 1976, the JECFA evaluated glycerine (glycerol) noting that it "*occurs naturally in fats and other substances which are in part made up of lipid complexes. Glycerol may be derived from natural sources, primarily triglycerides, or be synthesized by the hydrogenolysis of carbohydrate materials or from products such as propylene. Evidence is available to show that glycerol is metabolised in the body to form glycogen or provide a direct energy source. In addition, long-term studies are available to show that synthetically derived glycerols are biologically similar to naturally derived glycerol.*" The JECFA allocated an Acceptable Daily Intake (ADI) "not specified" but noted that, because certain butanetriols can be contaminants of glycerol produced from the hydrolysis of carbohydrates, there is a

[22] Now: Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES).

need to specify maximum levels of such contaminants (FAO/WHO, 1976). A specification of 0.2 % butanetriols was set by JECFA in 1976 (FAO/WHO, 2006). The CONTAM Panel noted that butanetriols do not occur during the biodiesel process as discussed in this opinion.

3.3. Use and toxicity of glycerine and crude glycerine from biodiesel as a feed component

Ruminants

Glycerine is a natural co-product of digestion in ruminants when dietary fats are fermented in the rumen. The rumen microbes adapt to glycerine feeding and most of the glycerine will be catabolised in a few hours.

In ruminants, the role of glycerine as a control adjuvant for ketosis and steatosis in **dairy cows** was explored already in the 1970s with concentrate mixtures containing 3 or 6 % glycerine (Fisher et al., 1971, 1973). The use of glycerine for dairy cows nutrition programs depend on the desired outcome. When fed, glycerine provides a supplement that is essentially “pure energy”. It enhanced fermentation characteristics and possible feeding efficiencies (Donkin et al., 2007; Hippen et al., 2008).

Kerley (2007) concluded that the use of glycerine from biodiesel in animal feed is limited by the feed used and acceptable supplement rate. According to this study, the energy value of this glycerine is similar to that of maize and supplementation rates of more than 20 % are possible. When fed to **steers** together with a low-starch concentrate, crude glycerine from biodiesel production (using vegetable oils as feedstock) at dietary inclusions up to 200 g/kg did not adversely affect nutrient digestibility (Cottrill et al., 2007).

Feeding trials have been performed in lactating dairy **cattle** by Donkin et al. (2007) using 99.5 % USP grade glycerine. The data indicate that glycerine is a suitable replacement for corn grain in diets for lactating dairy cattle and that it may be included in rations to a level of at least 15 % of dry matter without any adverse effects on milk production or milk composition. Precautions should be taken when introducing glycerine at higher levels to the diet, as approximately 7 days are required to adapt the rumen flora to glycerine feeding. The authors emphasised that these findings might not be applicable when crude glycerine derived from biodiesel production is used as a feed component.

AFSSA stated that no adverse effect could be observed when crude glycerine (containing more than 80 % glycerine and derived from vegetable oil) was given to **dairy cows** at up to 100 g/kg feed concentrate in a diet containing 50 % concentrates. It was stated also that since glycerine is a natural metabolite resulting from digestion, the harmlessness of the compound can be extrapolated to other ruminant species (AFSSA, 2007).

Monogastrics

Several studies have evaluated crude glycerine made from vegetable oil in diets for **poultry** and **swine**. Wagner (1994) performed feeding trials with glycerine in pigs (5 to 10 % glycerine added in the diet) and broilers (10 to 15 % glycerine added in the diet). Amounts of glycerine up to a maximum of 10 % were recommended by the authors for use in feed for broilers.

The utilisation of pure glycerine, as well as crude glycerines originating from two different technical processes of biodiesel production, was investigated in a feeding experiment using **boars.** Glycerine was included into the diets in amounts of 5 and 10 %. There were no differences seen between technical (crude) and pure glycerine concerning the feeding performance (Kijora, 1996).

Kijora et al. (1995, 1997) included crude glycerine (derived from biodiesel vegetable oil and ranging from 5 to 30 %) in the diets of **fattening pigs**. Daily weight gain, feed intake and feed conversion were measured. Glycerine in diets did not change the carcass and the meat quality. An inclusion level of glycerine of up to 10 % in the complete diet was recommended by the authors.

According to Poilvet (2005), the use of crude glycerine as a concentrate derived from vegetable oil added to the diet of sows during pregnancy increased the number of piglets at birth and improved their vitality. The amount of glycerine is not detailed in this publication. Studies on the effects of the addition of pure glycerine in pigs diets showed reduced growth and poorer feed:gain ratio when given at 10 % but not at 5 % of the diet (DM basis) (Della Casa et al., 2009).

AFSSA reported that 5 % (as a mean percentage derived from vegetable oil) crude glycerine in the diet of **pigs** results in an optimal energy recovery. Incorporation levels up to 10 % were tolerated without any adverse effects (AFSSA, 2007).

Feeding trials were performed in **broilers** (Simon et al., 1996, 1997) using crude glycerine (derived from vegetable oil and ranging from 5 to 25 % DM). The influence of glycerine supplementation on weight gain, feed intake and feed conversion ratio was beneficial when included in the complete diet at levels from 5 to 10 %. **Broilers** fed a 20 % glycerine diet had the same weight, feed intake or feed conversion as control diet (Campbell and Hill, 1962; Lin et al., 1976; Lessard et al., 1993; Simon et al., 1996).

Cerrate et al. (2006) evaluated crude glycerine derived from vegetable oil for biodiesel production as a feed ingredient for **broilers**. Their data indicate that glycerine from biodiesel can be a useful energy source in broiler diets at levels of 2.5 % or 5 %. Birds fed diets with 10 % glycerine consumed less feed and had significantly reduced body weights. The authors discussed that this finding could be due to reduced flow rate in the feeders used in the study and that use of levels higher than 5 % may be possible, but presumably not for all types of feeding systems. According to the authors, concerns remained regarding acceptable levels of residual amounts of potassium and methanol in crude glycerine from biodiesel production.

AFSSA reported that 5 % (as a mean percentage) crude glycerine in the diet of **poultry meat (chickens and turkeys)** delivers an optimal energy recovery. Incorporation levels up to 10 % had no adverse effects (AFSSA, 2007).

In **horses**, the effects of crude glycerine supplementation derived from vegetable oil on fluid and electrolyte balance during and after endurance rides have been investigated by Dusterdieck et al. (1999). Hypertonic oral electrolyte pastes, with or without glycerine, were given to Arabian horses performing an endurance ride. Adverse effects were not seen when rehydrating the animals with 3 g/kg body weight (b.w.) glycerine. Glycerine supplementation did not produce additional benefits to electrolytes. According to AFSSA, no adverse effects were seen in horses at feed inclusion levels corresponding to an intake of 1 g/kg b.w. (AFSSA, 2007).

Pet animals

Incorporation levels between 8 and 10 % crude glycerine can be tolerated in processed moist **pet food** (AFSSA, 2007).

Laboratory animals

The acute toxicity of glycerine (chemical grade) is very low. The Lethal Dose, 50 % (LD_{50}) after one single administration in **rats** is higher than 27,200 mg/kg b.w. The long-term toxicity of glycerine is also very low. In **dogs**, a no–observed-effect-level (NOEL) after a daily oral administration for 2 years of 8,000 mg/kg b.w. per day in males and 10,000 mg/kg b.w. per day in females was reported (AFSSA, 2007). Moreover, glycerine has neither carcinogenic nor affects reproduction effects in laboratory animals (rats, mice, rabbits) (AFSSA, 2007).

Aquatic species and environmental toxicity

The toxicity of glycerine for the environment is very low. The Lethal Concentration, 50 % (LC_{50}) after 24 hours exposure is higher than 10,000 mg/L for the crustacean *Daphnia magna*. The minimal toxic concentration TGK 3 % (Toxische Grenzkonzentration according to the Brinkmann-Kühn Assay) after 8 days exposure is higher than 10,000 mg/L for *Scenedesmus quadricauda* (algae) (AFSSA, 2007).

4. Methanol

The major impurity of biodiesel-derived glycerine is methanol, which is added to the production process to support trans-esterification of fatty acids. The methanol concentration in crude glycerine varies depending on the manufacturing process, but generally does not exceed 0.5 %. The current EU legislation intends to limit the amount of methanol in biodiesel (from different sources) to a maximum of 0.2 %.

4.1. Previous assessments on methanol impurities

In Germany, the positive list for straight feeding stuffs[23] sets a maximum level of 0.2 % (2,000 mg/kg) for methanol in crude glycerine.[24] In the United States, the FDA requires that the methanol content does not exceed 0.015 % (150 mg/kg) in the final animal feed (FDA, 2006). AFSSA concluded that a maximum value of 500 mg methanol/kg feed (0.05 %) at 88 % dry matter (DM) would be acceptable (AFSSA, 2007). These values are difficult to compare as they address the raw material, the finished feed or the finished feed at 88 % DM.

Considering the United States Environmental Protection Agency study on rats (US-EPA, 1986 cited in US-EPA, 1993; see section 4.2) suggesting a NOEL of 500 mg/kg b.w. per day, AFSSA introduced an uncertainty factor of 10 to account for possible species differences in order to derive a NOEL of 50 mg/kg b.w. per day for repeated oral administration to mammals and poultry.

4.2. Toxicokinetics and toxicity of methanol

4.2.1. Toxicokinetics

After oral exposure, peak serum levels of methanol occur within 30-90 min and methanol is distributed throughout the body with a volume of distribution of approximately 0.6 litre/kg (WHO, 1997). The serum half-life of methanol in mammals including humans varies between 142 and 213 minutes (Jones, 1987). Methanol metabolism occurs in the liver and involves 3 sequential oxidative steps to formaldehyde, formic acid and carbon dioxide. The initial step involves oxidation to formaldehyde by hepatic alcohol dehydrogenase (CYP2E1) in primates including humans and in most food producing animal species (Sweeting et al., 2010). The second step involves oxidation of formaldehyde to formic acid/or formicate, depending on the pH, by formaldehyde dehydrogenase. In the final step, formic acid is detoxified to carbon dioxide by folate-dependent reactions (WHO, 1997).

In ruminants, methanol is degraded to formaldehyde and subsequently metabolised by the rumen flora (pre-systemic elimination).

[23] http://www.dlg.org/fileadmin/downloads/fachinfos/futtermittel/positivliste/positivelist_en_8.pdf.
[24] Anonymous, 2007, cited in Hansen et al., 2009.

Based on these data, it is highly unlikely that the low exposure rate to methanol as an impurity of glycerine derived from biodiesel production causes any residues (parent compound and its metabolites) in animal tissues.

4.2.2. Toxicity

Laboratory animals

The acute toxicity of methanol is low. LD_{50} values after single oral administration in rats, mice, rabbits, guinea pigs, dogs and cats vary between 6,000 and 14,000 mg/kg b.w. (AFSSA, 2007).

Experimental data on long-term oral methanol toxicity are limited. A sub-chronic oral toxicity study in Sprague-Dawley rats was conducted by US-EPA in order to set a chronic oral reference dose (RfD) for methanol (US-EPA, 1986). Animals (30 males and females per dose) were exposed by gavage to 0, 100, 500, or 2,500 mg methanol/kg b.w. per day. There were no differences between dosed animals and controls in body weight gain, food consumption, and gross or microscopic findings. Elevated liver enzyme activities in the serum and an increase (not statistically significant) in liver weight in both male and female rats suggested possible treatment-related effects in the highest methanol dose group. The NOEL was considered to be 500 mg/kg b.w. per day, to which US-EPA applied an uncertainty factor of 1000 to set an RfD of 0.5 mg/kg b.w. per day in 1988 (US-EPA, 1993).

4.3. Exposure of animals to methanol as impurity of biodiesel glycerine

In the following text, exposure rates to methanol were calculated assuming an inclusion rate of glycerine of 10 % (with a methanol impurity of 0.5 %) corresponding to final concentration in feed of 500 mg/kg for monogastric animal species. For ruminants, an inclusion rate of up to 15 % of the total ration (DM) was considered. These inclusion rates correspond to the levels of glycerine used is most of the above-mentioned feeding trails. Exposure would be even lower at the recently recommended level of 0.2 % (see section 2).

Ruminants

For a 600 kg adult dairy cow, consuming 20 kg DM per day (50 % in the form of concentrate containing 15 % glycerine with a methanol impurity of 0.5 %), a daily methanol intake of 12.4 mg methanol/kg b.w. per day can be calculated. This concentration will be tolerated and degraded and/or converted into carbon dioxide and water by the rumen flora.

Pigs

In pigs, a daily intake of 10 mg methanol/kg b.w. per day can be calculated for a pig of 100 kg, consuming 2 kg feed containing 10 % glycerine with a methanol impurity of 0.5 % per day (1,000 mg methanol per day); for a 250 kg lactating sow, eating 6 kg feed per day (3,000 mg methanol per day) would correspond to a daily intake of 12 mg methanol/kg b.w. per day, hence remaining below the NOEL of 500 mg/kg b.w. for rats derived by AFSSA from the EPA study.

In a study from Hansen et al. (2009), **growing-finishing pigs** were fed with diets containing different percentages of crude glycerine (ranging from 4 to 16 %) from the production of biodiesel with different feedstocks. No clinical symptoms were observed related to methanol toxicity even at the highest possible percentage of methanol (0.29 % methanol or 2,900 mg/kg in the diet). The authors noted that a significant proportion of the methanol evaporated from the diets.

In poultry, the exposure of a 1.1 kg 4 week-old turkey (or broiler), eating about 0.11 kg feed per day, would be approximately 0.055 g methanol per day (55 mg methanol/kg b.w. per day). The daily exposure of an 8 week-old turkey (2.2 kg) equals to about 25 mg methanol/kg b.w. per day. This means that for a 1.1 kg turkey (or broiler) the levels of exposure will reach the NOEL derived by AFSSA from the EPA study and remain below the derived NOEL for other animals.

In a study by Cerrate et al. (2006), **broilers** were fed with glycerine from biodiesel production. According to these authors, the quality of the glycerine is a concern, but acceptable levels for residual methanol or other contaminants were not determined.

Dogs

For a dog of 15 kg, eating 250 g biscuits, the methanol intake will correspond to 125 mg methanol per day, i.e. 8.5 mg methanol/kg b.w. per day remaining below the NOEL of 500 mg/kg b.w. for rats derived by AFSSA from the EPA study.

Aquatic species and environmental toxicity

The toxicity of methanol for the environment is very low. The Effective Concentration 50 % (EC_{50}) after 96 hours exposure is higher than 100 mg/L for *Daphnia magna* (microshellfish). The LC_{50} after 48 to 96 hours exposure ranged between 100 and 15,000 mg/L for the main fish species (AFSSA, 2007).

5. Sodium

In addition to methanol, sodium is considered to be a significant impurity in the glycerine fraction obtained from the biodiesel production. Like methanol, sodium is added to the production process, mainly in the form of sodium hydroxide, as a catalyst in the alkalisation and trans-esterification process. In the crude glycerol, sodium impurities will be found mainly in the form of sodium chloride (AFSSA, 2007).

Only few data are available on the sodium content of glycerine from biodiesel production. A comparison of different feedstocks and the resulting sodium concentration of glycerine has been published by Thompson and He (2006). While for most materials the sodium concentration varied between 1.07 and 1.2 % (10,700 - 12,000 mg/kg), it may reach in certain conditions 1.4 % (14,000 mg/kg).

5.1. Previous assessments on sodium

A risk assessment on sodium was performed in 2005 by EFSA's Scientific Panel on Dietetic Products, Nutrition and Allergies (EFSA, 2005). The Panel estimated that the dietary intake of sodium in humans in Europe varies between 4,500 and 11,000 mg/day. No tolerable upper intake could be established for sodium from dietary sources.

5.2. Toxicity of sodium

For sodium chloride, the oral LD_{50} in rats is 3,000 mg/kg and in mice is 4,000 mg/kg (EGVM, 2003; RTECS, 1998).

In domestic animals, salt toxicity is generally only a problem in the absence of sufficient amount of drinking water (see for example feeding of milk replacers or whey without sufficient water supplies). Maximal concentrations of salt that can be present in animal diets without adversely affecting intake, growth or performance of the animals were defined by the National Research Council NRC (2005). In farm animals, ruminants in particular, 1 g/kg b.w. is the maximum tolerable sodium intake for a 600 kg lactating dairy cow. The maximum tolerable salt load for pigs is also 1 g/kg b.w. The maximum tolerable amount of salt that can be fed to poultry without affecting feed intake is 1.5 g/kg b.w. In domestic animals, studies on cats show that they can safely consume about 1.37 g salt/kg b.w., while dogs can safely consume 0.64 g salt/kg b.w.

5.3. Exposure of animals to sodium as impurity of biodiesel glycerine

The exposure to sodium as impurities (1 %) in the glycerine obtained from the biodiesel production process included in the diet of farm animals (10 % of the total ration (as DM) in monogastric animals and 15 % of the total ration (as DM) in ruminants) would increase the sodium intake by up to 1 and 1.5 g/kg feed material respectively.

Ruminants

For a 600 kg adult dairy cow, consuming 20 kg DM per day containing 1.5 g sodium/kg feed material, the daily sodium intake would increase by up to 50 mg sodium/kg b.w. per day. According to the values mentioned above, this increase is not expected to be a health concern.

Pigs

In pigs, the daily sodium intake will increase by 20 mg sodium/kg b.w. for a pig of 100 kg, consuming 2 kg feed containing 1 g sodium/kg feed material. For a 250 kg lactating sow, eating 6 kg feed per day (6 g sodium per day) would correspond to an increase in daily sodium intake by up to 24 mg sodium/kg b.w. According to the values mentioned above, this increase is not expected to be a health concern.

Poultry

In poultry, the daily sodium intake for a 1.1 kg 4 week-old turkey (or broiler), eating about 0.11 kg feed per day, would increase by up to 110 mg sodium per day (100 mg sodium/kg b.w. per day). The daily sodium intake for an 8 week-old turkey (2.2 kg) would increase by up to 50 mg sodium/kg b.w. According to the values mentioned above, this increase is not expected to be a health concern.

However, Cerrate et al. (2006) reported experiments in which broilers were fed with glycerine from biodiesel production. According to these authors, the quality of the glycerine is a concern as residual levels of potassium (from potassium salt catalysts) may result in problems with wet litter or imbalances in dietary electrolyte balance.

Dogs

For a dog of 15 kg eating 250 g biscuits (dry feed), the sodium intake will increase by up to 250 mg sodium per day, i.e. 16.7 mg sodium/kg b.w. per day. According to the values mentioned above, this increase is not expected to be a health concern.

6. Other impurities

Besides methanol and sodium chloride, common impurities of crude glycerine are residual amounts of the catalyst, used for the trans-esterification process. Although other contaminants of biodiesel have been investigated (Monteiro et al., 2008), only very limited data on possible impurities of the crude glycerine fraction (see previous sections), other than methanol and sodium, could be traced. In some processes sodium salts are replaced by potassium salts and methanol may be replaced by ethanol if (recycled) waste cooking oil or fat are used as an alternate feedstock for biodiesel production (Chhetri et al., 2008). No data are available on the residual amounts of potassium, ethanol and other production aids in the crude glycerine fraction.

As stated above and in compliance with EU legislation, ABP of all risk categories may be used as well as feeding stock for biodiesel production. The physico-chemical properties of glycerine suggest that many other undesirable compounds which may be present in the input material, such as heavy metals, mycotoxins or residues of veterinary medicinal products, may co-elute into the crude glycerine fraction. While the aggressive chemical processes involved in the trans-esterification are likely to degrade many of those possible contaminants, no data are available regarding this type of contamination of crude glycerine obtained from biodiesel production.

7. Risk characterisation

7.1. Animal health risk characterisation

Data obtained from previous assessments and from the literature suggest that an inclusion of crude glycerine as a feed compound of up to 15 % of the total ration (as DM) in ruminants and 10 % of the total ration (as DM) in monogastric animals is without adverse effects. These conclusions are based on feeding experiments conducted with crude glycerine, derived from biodiesel production using vegetable oils. Some older studies were conducted with technical glycerine. The purity of the different forms of glycerine is not reported in detail.

Regarding the impurities of crude glycerine, the CONTAM Panel concluded that it is unlikely that the amount of methanol present in the glycerine of up to 0.5 % in the feed of monogastric animals (corresponding to 500 mg/kg feed at the inclusion rate of 10 %) could result in adverse health effects The CONTAM Panel also concluded that it is unlikely that the amount of methanol present in the glycerine of up to 0.5 % in the feed of ruminants (corresponding to 750 mg/kg feed at the inclusion rate of 15 %) could result in adverse effects. These conclusions are based on the available reports in the literature, which also indicate that some of the residual methanol will evaporate during feed processing and storage. The CONTAM Panel acknowledges that the level of 0.2 % methanol in crude glycerine from biodiesel production as proposed for the envisaged EU Catalogue of Feed Materials will provide an additional margin of safety for animal health (for details see section 2).

Likewise, the increase in sodium intake when including glycerine from the biodiesel production with a sodium content of 1 % will not be a concern for animal health.

In contrast, there are concerns regarding potential higher residual amounts of ethanol and potassium that might be present in glycerine that is derived from biodiesel production using other feedstocks than only vegetable oils. The use of diverse feedstocks, including animal fats, requires amendments of the technical process of biodiesel production and may therefore result in a different composition of catalyst residues. Even more importantly, due to the physico-chemical characteristics of glycerine and methanol or ethanol, crude glycerine derived from other processes than vegetable oil trans-esterification, may also contain diverse undesirable substances that are present in the feedstock materials. Despite the fact that this risk has also been noted in previous assessments and publications, no data on the amount of these substances could be traced.

7.2. Human risk characterisation

Glycerine is a physiological substance, and it is highly unlikely that addition of glycerine to animal diets at the inclusion levels tolerated by the animal without loss in performance would result in any harmful residues in the animal or in animal derived products. In addition, glycerine is permitted as a food additive (see section 3).

Crude glycerine derived from biodiesel production using vegetable oils as feedstock material can contain residual amounts of methanol and sodium. Feeding trials indicated that some of the residual methanol will evaporate during feed processing and storage. In addition, previous investigations regarding the serum half-life of methanol in mammals (including humans) indicated that the half-life of methanol varies between 142 and 213 minutes (Jones, 1987). No persistent residues have been identified. Hence, it is unlikely that residual amounts of methanol will occur in foods of animal origin. Residual amounts of sodium in crude glycerine will enter the electrolyte pool of the animal's body and will be rapidly excreted, making unlikely a contamination of product from animal origin.

8. Uncertainties

The typical composition of glycerine derived from oils and fats (others than vegetable oils), after the biodiesel production process, has not been investigated in detail. The CONTAM Panel concluded that this crude glycerine may contain an as yet unknown quantity of processing aids (catalysts) and contaminants of the feedstock material. In particular the use of Category 1 ABP material, defined as entire bodies or parts of animals or products of animal origin not intended for human consumption, which may comprise products derived from animals to which prohibited substances have been administered and products of animal origin containing residues of environmental contaminants and other undesirable substances, remain of concern unless it is proven that the rigid chemical processes involved in the trans-esterification of the feedstocks in the biodiesel production indeed inactivate these contaminants.

CONCLUSIONS AND RECOMMENDATION

CONCLUSIONS

- The major co-product of biodiesel production is crude glycerine. Crude glycerine may contain a variable amount of impurities resulting from the biodiesel process and is used without further purification as a feed material in the diet of monogastric animals as well as in ruminants.

- Methanol is the major impurity of crude glycerine derived from biodiesel production using vegetable oils. In commonly applied technical processes, the residual methanol concentration in the crude glycerine fraction is generally below 0.5 %. In terrestrial animal species, this level of contamination does not represent a risk for animal health at a total inclusion level of 10 % in the diet of monogastric animals and 15 % crude glycerine in the diet of ruminants.

- In common technical processes, using vegetable oils as input (feedstock) for biodiesel production, sodium (originating from sodium hydroxide in the alkalinisation process) is found in the crude glycerine fraction up to a level of 1 %. At the given inclusion levels of 15 % glycerine for ruminants and 10 % for monogastric animals, these amounts contribute only marginally to the daily sodium intake of the animals. This increase is not expected to be a concern for animal health.

- The ultimate composition of crude glycerine, obtained as a co-product from biodiesel production, depends on the technical process (selection of input material) and the applied technical procedure, including the use of methanol (and in some cases ethanol) and other catalysts (acids or bases). No data are available regarding the levels of possible contaminants of the crude glycerine obtained from biodiesel production processes using other materials than vegetable oils used for human consumption.

- Considering the physico-chemical properties of the glycerine and the glycerine-methanol or glycerine-ethanol mixture, it cannot be excluded that other undesirable compounds present in the input material are co-eluted into the crude glycerine. In particular, this applies to the use as feedstock material of animal by-products classified as category 1 due to a non-compliant presence of undesirable or unauthorised substances.

- As crude glycerine derived from biodiesel production using input materials others than vegetable oils intended for human consumption may contain residues of environmental contaminants and other substances, it remains of concern to human and animal health unless it is proven that the chemical processes involved in the trans-esterification of the feedstock in the biodiesel production inactivate these abiotic (chemical) contaminants.

RECOMMENDATION

The CONTAM Panel recommends the collection of data on the presence of impurities and contaminants in crude glycerine from biodiesel production that is used as an animal feed component.

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ABBREVIATIONS

ABP	animal by-products
ADI	acceptable daily intake
AFSSA	Agence française de sécurité sanitaire des aliments
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail
BIOHAZ Panel	Panel on Biological Hazards
b.w.	body weight
CONTAM Panel	Panel on Contaminants in Food Chain
DM	dry matter
EFSA	European Food Safety Authority
FAO/WHO	Food and Agriculture Organisation/World Health Organization
FDA	United States Food and Drug Administration
GRAS	generally recognised as safe
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC_{50}	lethal concentration, 50 %
LD_{50}	lethal dose, 50 %
NOEL	no-observed-effect-level
RfD	reference dose
US grade	U.S. Pharmacopoeia