

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

CHRONIC TOXICITY OF METHANOL - INTRODUCTION

Background and introduction

1. It is well established that acute exposure to high levels of methanol results in a variety of toxic damage, notably to the nervous system. In particular, methanol exposure can result in a range of ocular damage as a result of tissue hypoxia. Oral exposure to high levels of methanol can occur through self-poisoning or from consumption of products such as counterfeit or illegally distilled alcohol but inhalation of methanol through industrial exposure may also occur.
2. Methanol is also present at low levels in a variety of foods notably fruits and vegetables where it is released from pectin as well as being present in a free form. It is also released following the breakdown of the sweetener aspartame. It is generally considered that dietary methanol would not result in adverse effects since the body can safely metabolise a low level of methanol.
3. Methanol and ethanol are both metabolised by alcohol dehydrogenase (ADH). The affinity of ADH for ethanol is much higher than for methanol and the ability of ethanol to out-compete methanol is exploited in the treatment of clinical methanol poisoning.
4. It has been argued by some that naturally-occurring methanol is always accompanied by ethanol and thus would not have any adverse effects on health, whereas the methanol released from additives would not be accompanied by ethanol and therefore would be potentially adverse.

Current FSA advice and available data

5. The advice currently given by the FSA is that dietary methanol from any source is not of concern, and we would further advise that while ethanol is metabolised in preference to methanol, this delays rather than prevents the metabolism of methanol to formaldehyde, formate and ultimately CO₂ and thus does not significantly influence the effects of dietary methanol.
6. The following introductory paper presents information on methanol pharmacokinetics, sources of chronic methanol exposure, data on acute and chronic effects in humans and laboratory animals and considers the effects of ethanol on methanol toxicity. It is intended to provide a summary rather than full review of this issue.
7. The COT is asked to comment on the current FSA guidance and on whether there are sufficient data available to make a review of chronic methanol toxicity worthwhile, with either a view to establishing a TDI, or, providing some other form of guidance.

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Dietary sources of methanol, ethanol and formate.

8. Methanol occurs naturally in food, notably in fresh fruits and vegetables and their juices. It occurs as free methanol, methyl esters of fatty acids or methoxy groups on polysaccharides such as pectin. Thus there is “existing” (i.e. free) methanol in the fruit or fruit juices plus the “potential” (i.e. releasable) methanol that is made available as pectin is broken down during digestion in the gastrointestinal tract, meaning that the potential methanol intake from the diet could be higher than analysis of the methanol content of individual foodstuffs might suggest.

9. In fruit juices, the methanol content ranges from 1- 640 mg/L with an average of 140 mg/L. Methanol may also occur in dried legumes at levels of 1.5-7.9 mg/kg. Other sources of dietary methanol include filbert nuts (a species of hazelnut) and vegetables such as potatoes, onions, Brussels sprouts, celery, and parsnips. Total exposure to methanol from natural sources is uncertain, but intakes of 10.7 and 33 mg/day have been reported in US consumers (Magnuson *et al*, 2007). However, this is likely to be a significant underestimate since the analysis concerned did not include data from processed foods or from food sources such as potatoes or onions or the methanol released by fruits and vegetables. It has been estimated that humans may produce approximately 1000 mg methanol from fruits and vegetables every day (Taucher *et al*, 1995); riper fruit was found to release more methanol than unripe fruit.

10. Methanol also occurs at low levels in alcoholic drinks. Levels of 6-27 mg/L have been measured in beer, 96-321 mg in wine and 10-220 mg/L in distilled spirits (WHO, 1997). The presence of methanol in distilled spirits is related to the pectin content, which is broken down during production leading to the release of methanol. Since 2008 there has been a direct EU limit on methanol in vodka of 10 grams per hectolitre of 100 % vol. alcohol (which equates to 100 mg alcohol per litre). Data from incidents show that some illegally distilled or counterfeit alcoholic drinks contain much higher levels of methanol.

11. The artificial sweetener aspartame (see Fig 1) is a methyl ester of a dipeptide consisting of aspartic acid and phenylalanine. It is rapidly broken down and is thought to release 10% methanol by weight. The ADI for aspartame is 40 mg/kg bw/day, resulting in a maximum potential exposure of 4 mg methanol/kg bw/day (or 240 mg/day in a 60 kg adult) from this source. Thus, the amount of methanol released from dietary exposure to aspartame is less than the potential dietary exposure to methanol from fruits and vegetables and their juices and from alcoholic beverages. For example, a serving of tomato juice provides about 6 times more methanol than an equivalent volume of beverage sweetened 100% with aspartame (WHO, 1997).

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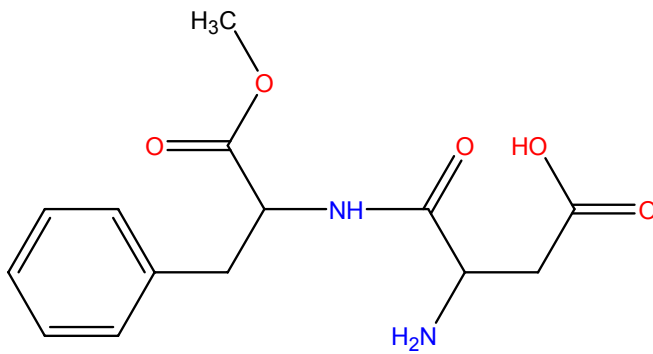


Figure 1. Aspartame (6-methyl-1,2,3-oxathiazine-4(3H)-one-2,2-dioxide salt of L-phenylalanyl-2-methyl-L- α -aspartic acid).

12. Ethanol is also naturally present in fruits and fruit juices with levels of 90-900 ppm being measured in fresh, canned and canned then stored juices compared to methanol which was present at 10-80 ppm (Lund *et al*, 1991). Other foodstuffs too can contain varying contents of ethanol if alcoholic beverages have been used in their preparation.

13. Dietary formate exposure may occur through consumption of honey, fruit syrups and roasted coffee. Calcium formate supplements have been investigated as a readily bioavailable source of calcium (Altaweel *et al*, 2009).

Pharmacokinetics of methanol

14. Methanol is readily absorbed by ingestion, inhalation and dermal exposure. Methanol is absorbed orally within 30-60 minutes, depending on the presence or absence of food. After uptake the majority of the methanol (96.9%) is converted to carbon dioxide in the liver, with a small fraction being excreted directly in the urine or by the lungs.

15. Methanol is oxidised sequentially to formaldehyde, then to formic acid or formate (depending on the pH) and finally to carbon dioxide. In humans and non-human primates, the oxidation of methanol to formaldehyde is mediated by ADH. In other mammals, the reaction is mediated by catalase but the conversion rates are similar. The oxidation of formaldehyde to formate is mediated by several enzyme systems including formaldehyde dehydrogenase.

16. Formate is then oxidised to carbon dioxide through the action of formyl-THF synthetase, where formic acid combines with tetrahydrofolic acid (THF) to form 10-formyl-THF which is subsequently converted to carbon dioxide by formyl-THF-dehydrogenase (Cruzan, 2009). Formate oxidation to carbon dioxide is variable between species, which determines sensitivity to methanol toxicity (it is twice as slow in humans and non-human primates compared to rats). This variability is due to the availability of folate. In humans and monkeys, THF levels are 60% and 16-26% of those found in rat and mouse liver respectively. Formyl-THF-dehydrogenase levels

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are also reduced in humans and monkeys, being 25% and 37% respectively of the levels found in rat liver.

17. Ethanol and methanol are cleared at a constant rate regardless of dose. Metabolic clearance of methanol (although saturable) is much faster than the excretion of unchanged methanol. Once metabolic clearance is saturated unchanged methanol is still excreted at a constant rate, but because of the saturation of metabolic clearance formate can accumulate, resulting in toxicity. However, there is an amount of methanol and/or ethanol that can be safely ingested and metabolised without symptoms of toxicity.

18. Clearance of methanol is slow, particularly when compared to ethanol. Half times of 2.5-3 hours are reported for doses of less than 0.1 g/kg methanol given to human volunteers but increasing to 24 hours or more for doses greater than 1g/kg (WHO, 1997). Stegink *et al* (1981) reported that methanol released from high (abuse) doses of aspartame – 80 mg/kg bw cleared with half times of 2.5 to 3h in human volunteers.

Mechanism of toxicity

Symptoms

19. As noted above, humans and non-human primates are more sensitive than rats to methanol toxicity due to their slower conversion of formate to carbon dioxide.

20. In species that metabolise formate poorly such as primates, acute and short term methanol toxicity is characterised by formic acidaemia, metabolic acidosis, ocular toxicity, nervous system depression, blindness, coma and death. The consequences of longer term exposure to lower levels of methanol are a range of ocular effects. In species which metabolise formate readily, central nervous system (CNS) depression is usually the cause of death.

21. In humans, the signs and symptoms of methanol poisoning, which may appear after an asymptomatic period of 12-24 hours, include visual disturbances, nausea, abdominal and muscle pain, dizziness, weakness and disturbances of consciousness ranging from coma to chronic seizures. The ocular effects develop 12-48 hours after methanol ingestion and range from, mild photophobia, misty or blurred vision to significantly reduced visual acuity and complete blindness. Visual disturbances have been reported in workers exposed to 1500 mg/m³ (1200 ppm) methanol or greater. However, certain features of methanol damage to the visual system such as pallor of the optical disc are end stage symptoms of irreversible damage and may appear 1-2 months after acute exposure.

22. Toxicity becomes apparent when formate production continues to exceed clearance. Formate inhibits the mitochondrial enzyme cytochrome oxidase, resulting in tissue hypoxia; acidosis and other clinical symptoms may also occur if the formate load is high (Liesivuori and Savolainen, 1991). The ocular effects associated with methanol poisoning appear to be due to hypoxia in areas of the cerebral and distal optic nerve circulations.

Effects due to acute or chronic toxicity

Humans

23. The vast majority of human data relate to acute or short term toxicity. The minimum lethal dose is 0.3 to 1 g/kg bw (20 to 60 g or 25-75 mls/person in a 60 kg adult) (WHO, 1997). The minimum dose associated with ocular toxicity is unclear but may be 10 mls (8 g or 133 mg/kg bw) (Vale, 2007). There is wide inter-individual variability in the toxic dose. The most important determinants of susceptibility to methanol toxicity are concurrent ingestion of ethanol and folate status in the liver.

24. No adverse effects were apparent in 12 women given 1400 mg calcium formate supplements 3 times a day for 14 days (Altaweel *et al*, 2009). Study evaluations included physical and ocular examinations, extensive laboratory tests, serum calcium and formate levels.

25. Limited case reports suggest that extended exposure to methanol may cause effects qualitatively similar to those arising from acute exposure including, in some cases, CNS and visual disorders. For example, in workers regularly using spirit duplicators (which used a 99% methanol duplicator fluid), symptoms such as dizziness, headaches, nausea and visual disturbances were reported (Frederick *et al*, 1984). Measurements showed that the airborne concentration of methanol vapour ranged from 365-3080 ppm, with 15/21 measurements exceeding the NIOSH (National Institute for Occupational Health and Safety) recommended 15 minutes maximum exposure limit of 800 ppm. Reviewing this data, WHO (1997) concluded that adverse effects may become apparent where the threshold limit value of 260 mg/m³ (200 ppm) methanol is exceeded. The symptoms reported by Frederick *et al*, (1984) were noted to be similar to, but less severe than, the symptoms associated with acute methanol poisoning

Animals

26. Most animal studies of methanol have been conducted using inhalation exposure and are of short duration. In the less sensitive species such as rats, symptoms of upper respiratory irritation were apparent at doses of 5000 ppm methanol for 6 h/day, 5 d/week for 4 weeks. No signs of pulmonary toxicity were seen in rats exposed to 10,000 ppm for 6 h/day and 5 d/week for 4 weeks. In monkeys given >3000 mg/kg methanol by gavage, ataxia, weakness and lethargy were seen with primates within a few hours, this disappeared within 24 hours, but was followed by transient coma in some animals.

27. The inhalation of methanol by pregnant rodents is associated with a range wide of concentration dependent teratogenic and embryo lethal effects. In macaques exposed to methanol vapour (200-1800 ppm for 2.5 h/day, 7 days/ week from prior to breeding and throughout pregnancy) the length of pregnancy was reduced by 6-8 days compared to controls, suggesting a possible effect on the foetal neuro-endocrine system affecting the timing of birth (Burbacher *et al*, 2004). The methanol treated animals experienced more complications such as uterine bleeding and

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prolonged, unproductive labour but this was not statistically significant. No other data on primates are available.

28. Methanol was reported to cause cancer in a variety of organs in rats given 500, 5000 or 20,000 ppm methanol in drinking water from 8 weeks until spontaneous death (Soffritti *et al*, 2002). The differences observed between treated and control animals were: a dose-related increase of total malignant tumours in treated males and females; a dose-related increase of carcinomas of the head and neck, mainly in the ear ducts in males of the treated groups and females in the 5000 and 20,000 ppm groups; a statistically significant increase ($P < 0.01$) of testicular interstitial cell hyperplasias and adenomas in the top dose group; an increase in sarcomas of the uterus in the top dose group; a dose-related increase in osteosarcomas of the head in males and female of the treated groups; and a dose-related increase in hemolymphoreticular neoplasias in males and females in the treated groups. Limited other data are provided in this paper and information on historical controls and on survival are not included. Methanol carcinogenicity was recently reviewed by Cruzan (2009) who obtained additional unpublished data on the Soffritti study and pointed out that there was excessive early mortality and lung pathology in this study. He also noted that the diagnosis of some of the pathological lesions described in this and studies of other chemicals conducted by the same group, was not confirmed when a selection of pathology slides from one of these studies were subsequently reviewed by an independent panel. Cruzan (2009) also reviewed an unpublished inhalation study of methanol in rats which was commissioned by the Japanese New Energy Development Organisation. Taking into account the data from the inhalation and published genotoxicity studies, it was concluded that it was unlikely that methanol would be carcinogenic in humans.

Methanol and ethanol

29. As noted previously, where ethanol and methanol are consumed together, the ethanol is preferentially catabolised due to its greater affinity for the enzyme ADH (approximately 20:1) with the methanol being catabolised thereafter. In this situation, the unmetabolised methanol persists in the blood stream, declining only gradually through urinary and pulmonary excretion.

30. In the clinical situation, a constant infusion of much higher levels of ethanol is used as treatment for methanol poisoning. The basis for using a constant ethanol dosing rather than a single bolus dose is that the excess of ethanol maintains the competitive inhibition of methanol metabolism and prevents the production of excess formate which cannot be further metabolised and thus accumulates. However, whilst this inhibition is occurring, the unchanged methanol is still excreted at a constant rate and thus levels gradually decrease, eventually falling to a level where the rate of metabolism would not lead to the build up and toxicity of formate. If a single bolus dose of ethanol were used, the metabolism of methanol would not be inhibited for sufficiently long to allow methanol levels to fall, and once the bolus ethanol dose was cleared, methanol would be metabolised with sufficient methanol remaining, to allow the build up of formate and the consequent toxic effects.

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31. It has been argued that the methanol in fruit and vegetables is not of concern since it is always accompanied by ethanol which acts as a “natural antidote”, by out-competing ADH and preventing the production and accumulation of formate, whereas when the methanol is released from aspartame where there is no ethanol it could result in adverse effects since methanol metabolism and formate production would proceed uninhibited.

32. However, assuming the presence of ethanol was relevant at these low levels (ie that there was insufficient ADH to metabolise both) it would only delay methanol metabolism, resulting in the temporary persistence of unchanged methanol, but then the subsequent metabolism to formate, with only a small decline in methanol levels due to the excretion of un-metabolised methanol. There is no evidence that there would be insufficient capacity to metabolise the formate produced from dietary methanol to carbon dioxide and thus formate would not be expected to accumulate. This would still apply whether methanol was in the presence of ethanol or not.

Safe levels of exposure to methanol

33. A TDI or similar for oral methanol has not been established

34. There are occupational limits based on inhalation exposure, these have been adapted to estimate how much methanol can be safely ingested over a specified period (this assumes that inhaled and ingested methanol are comparable, as both are readily and rapidly absorbed) (Paine and Dayan, 2001). In the UK the occupational limit is 260 mg/m³ and would represent exposure to 2600 mgs methanol in an 8 hour period. The maximum permitted levels in the workplace are typically 10,000 times higher than the levels in rural (0.8 ppb , 0.001 mg/m³) and urban air (30 ppb , 0.04 mg/m³)

35. Based on data from volunteer studies and the occupational limits, Paine and Dayan (2001) suggest a dose of 2 g/day methanol can be considered safe and 8 g/day as toxic in the context of alcoholic drinks. This incorporates a safety factor of 4. It was considered that the ethanol content of the drinks was not relevant to setting a safe level for methanol, as although the ethanol would delay methanol metabolism, it would not be delayed sufficiently for methanol levels to drop significantly. This level is considerably in excess of likely dietary intakes from either fruit and vegetables and their juices or from additives, with the additional reassurance that the methanol released from dietary sources is likely to occur over a longer time period than that from alcoholic drinks.

ADI for aspartame and methanol

36. The ADI for aspartame is based on animal data. When the ADI for aspartame was established by the COT in 1992 (following initial approval in 1982), the possible effects of methanol as a breakdown product were discussed. It was noted that “*The amount of methanol contributed to the diet by aspartame is very low compared with that from other foods eg one pint of orange juice would provide more methanol to a three year old child than a 40 milligram per kilogram bodyweight (mg/kg bw) bolus dose of aspartame*” (COT, 1992). Subsequent reviews by the Scientific Committee

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on Food and the European Food Safety Authority have not specifically mentioned this issue.

Summary and discussion

37. Methanol toxicity occurs when the formation of formate from methanol metabolism continues to exceed the oxidation of formate to carbon dioxide by folate dependent clearance mechanisms. However, a small quantity of methanol can be safely handled by the body.

38. Dietary methanol is not thought to be of concern, since at these low levels of exposure, there is no evidence that methanol metabolism would be saturated and thus that formate accumulation would occur.

39. It has been argued that the methanol in fruit and vegetables is not of concern since it is always accompanied by ethanol which would inhibit methanol metabolism, whereas when the methanol is released from the breakdown of aspartame where there is no ethanol it could result in adverse effects since methanol metabolism would proceed uninhibited. However, it seems likely that the ethanol would merely delay rather than prevent the conversion of methanol to formate and so would mean the effects of naturally occurring methanol would be comparable to that released from aspartame.

40. At present there is no TDI for methanol. However, the occupational exposure limits are equivalent to a methanol intake of approximately 2600 mgs/8 hours and it has been suggested that 2 g/day would be a safe daily dose for methanol in alcoholic drinks.

41. The advice currently given by the FSA is that dietary methanol from any food source is not of concern at realistic levels of intake, and that while ethanol is preferentially metabolised to methanol, when they are present together, this delays rather than prevents the metabolism of methanol to formaldehyde and ultimately CO₂ so the effects of methanol present in fruit juices or comparable products would not differ in toxic potential

Advice from the Committee

42. The COT are asked to comment on:

- (i) the current FSA advice
- (ii) whether there are sufficient data available to make a review of chronic methanol toxicity worthwhile, with either a view to establishing a TDI or, providing some other form of guidance.

**Secretariat
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