

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

COT STATEMENT ON THE EFFECTS OF CHRONIC DIETARY EXPOSURE TO METHANOL

Introduction

1. Methanol (CH₃OH) is a colourless, volatile liquid with a mild alcoholic odour when pure. It is miscible with water and organic solvents such as acetone¹.
2. Methanol is readily absorbed and distributed throughout the body. A small proportion of methanol is excreted unmetabolised in the urine and the breath. The majority of ingested methanol is sequentially oxidised to formaldehyde, then formate or formic acid, most of which is ultimately excreted as carbon dioxide. A small proportion of formate is excreted unmetabolised in the urine.
3. It is well established that methanol has serious acute toxic effects which occur at high levels of exposure, and arise from the accumulation of formate. In primates, formate binds directly to, and inhibits, cytochrome oxidase, leading to disruption of cellular function, notably in the nervous system, and subsequent damage. Metabolic acidosis also occurs. However, much less is known about whether there are chronic effects at lower levels of methanol exposure.
4. Methanol is produced endogenously and exposure also occurs from the diet. In addition, exposure to methanol may occur occupationally or from consumption of counterfeit or illegally distilled spirits.
5. The COT was asked to consider the effects of chronic oral methanol exposure in response to consumer concerns that methanol arising from the breakdown of the sweetener aspartame could be harmful.

Exposure to methanol

Dietary methanol

6. 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 from which it can be released by digestion. Pectin is broken down during digestion in the colon, from where methanol can be absorbed, meaning that the potential methanol intake from the diet is higher than analysis of the free methanol content of individual foodstuffs might suggest. The methanol concentration of fruit juices ranges from 1- 640 mg/L with an average of 140 mg/L¹. Concentrations of methanol in fresh orange and grapefruit juice were in

the range 11-80 and 12-60 mg/L respectively². Canning may increase the methanol content of fruit and juices by trapping the volatile components. In human volunteers, consumption of 10-15 g isolated pectin or of 1 kg apples (containing approximately 10 g natural pectin) induced a significant increase in methanol in the breath and, by inference, in the blood³. Consumption of 1kg apples was estimated to release 500 mg methanol. It has been estimated that humans may be exposed to approximately 1000 mg methanol per day from fruits and vegetables; riper fruit was found to release more methanol than unripe fruit.

7. 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 and estimates vary. Mean and 95th percentile intakes of 10.7 and 33 mg methanol/day have been reported in US consumers using Daily Intake via Natural Food Occurrence (DINFO) analysis (discussed in Magnuson *et al*, 2007⁵). However, this is likely to be a significant underestimate since the analysis did not include methanol intake from processed foods or from food sources such as potatoes or onions, or the methanol released by pectin breakdown in fruits and vegetables. Monte (1984)⁶ proposed that methanol exposure from natural sources is much less than 10 mg/day. This appears to be based on a value taken from a US EPA document “Multimedia Environmental Goals for Environmental Assessment”⁷, and it is uncertain how it was derived and whether it included methanol released from pectin as well as free methanol already present in foods.

8. Methanol also occurs at low concentrations in alcoholic drinks. Concentrations of 6-27 mg/L have been measured in beer, 96-321 mg/L in wine and 10-220 mg/L in distilled spirits¹. The presence of methanol in distilled spirits is related to the pectin content, pectin being broken down during production leading to the release of methanol. Since 2008 there has been an EU regulatory limit on methanol in vodka of 10 grams per hectolitre of 100 % vol. alcohol (i.e. 100 mg methanol per litre of alcohol, equivalent to 37 mg/L if the vodka contains 37% alcohol)⁸. Some illegally distilled or counterfeit alcoholic drinks have been found to contain much higher concentrations of methanol with drinks made from “industrial methylated spirits” containing 5%(v/v) methanol: 95 %(v/v) ethanol⁹.

Aspartame

9. The artificial sweetener aspartame is a methyl ester of a dipeptide consisting of aspartic acid and phenylalanine. It is rapidly broken down in the gastrointestinal tract by peptidases and esterases, and releases a maximum of 10% methanol by weight⁵. The Acceptable Daily Intake (ADI) for aspartame is 40 mg/kg body weight (bw), which would result in a maximum potential exposure of 4 mg methanol/kg bw/day (or 240 mg/day in a 60 kg adult) from this source. Surveys suggest that in the UK, the maximum exposure to aspartame is likely to be much lower than the ADI. For example, the estimated exposure from high level (97.5th centile) child consumption of diet soft drinks was 12 mg/kg bw aspartame¹⁰, though this does not take into account other sources of exposure. The maximum permitted concentration for aspartame in diet soft drinks is 600 mg/L¹¹. Thus, consumption of a 500 ml drink would result in exposure to a maximum of 300 mg aspartame or 30 mg of methanol (0.5 mg/kg bw in a 60 kg adult).

Dietary formaldehyde and formate

10. Formaldehyde is present in a number of foodstuffs such as meat, fruit and vegetables. Dietary formate exposure may occur through consumption of honey, fruit syrups and roasted coffee. Calcium formate has been investigated as a readily bioavailable source of calcium for use in dietary supplements¹². However, at present, this would not be a permitted source of calcium for food supplements in the EU¹³.

Endogenous methanol

11. Methanol is present in blood, urine, saliva and breast milk¹.

12. It has been suggested that 300-600 mg methanol/day can be generated as a product of intermediary metabolism (cited in Lindinger *et al*, 2007³). Axelrod and Daly (1965)¹⁴ described a methyltransferase enzyme system in the pituitaries of rats and rabbits which metabolised S-adenosylmethionine to methanol and S-adenosylhomocysteine. Later work^{15, 16, discussed 17} demonstrated that this enzyme, protein carboxymethylase (PCM), methylates the free carboxyl groups of proteins. PCM is ubiquitous in mammalian tissues with particularly high levels being found in endocrine glands and nervous tissue¹⁸. Methanol is formed as the end product of this reaction through the action of protein methylesterases.

13. Majchrowicz and Mendelson, (1971)¹⁹ reported that as a consequence of inhibition of its metabolism, the mean blood methanol concentration in a group of alcoholic subjects increased from 20 mg/L following an alcohol-free week, to 270 mg/L after they had been allowed to drink freely for 11 days, at which time blood ethanol concentrations were between 1500 and 4500 mg/L. The experiment was conducted in a residential facility and the volunteers received a standardised diet. The drinkers consumed either grain alcohol or bourbon which contained 1 and 48 mg/L methanol respectively. The authors did not attempt to calculate how much methanol was being produced, but it could be estimated that blood concentrations increased by $250/11 = 23$ mg/L/day; assuming total body water of 49 L, this would imply total exposure to 1.1 g/day methanol from endogenous production and external exposure. The pattern of methanol accumulation was the same in both groups. The methanol content of the bourbon was estimated to produce a concentration of 0.6 mg/L in the body water of a 70 kg male.

14. A value of 0.13-1.03 g/day for methanol exposure from endogenous and exogenous sources was estimated by Dhareshwar and Stella (2007)²⁰.

Endogenous formic acid and formaldehyde

15. Metabolic processes such as O- and N-demethylation reactions of endogenous and exogenous molecules generate formaldehyde which is then metabolised to formic acid²⁰. Total formaldehyde content in the body has been estimated to be 2.6 mg/kg bw²¹ and it can be assumed to be distributed across all aqueous body fluids due to its high aqueous solubility²⁰.

16. The value of 0.13-1.03 g/day methanol exposure estimated by Dhareshwar and Stella (2007)²⁰ was calculated to result in daily formaldehyde exposure from methanol of 0.11-0.96 g/day. However, this appeared to account for only a small fraction of the formaldehyde in systemic circulation. Using a value of 49 L for total

body water (0.8 L/kg bw for a 60 kg adult) and an equilibrium concentration of 2.6 mg/L (the background tissue formaldehyde concentration), the total formaldehyde content in the body can be estimated to be 122.5 mg²⁰. However, to maintain the equilibrium concentration of 2.6 mg/L, it was calculated that the daily turnover of formaldehyde would be 31-59 g/day, meaning that external sources of formaldehyde, including methanol, account for only 1-2% of the total daily turnover.

17. Formic acid is produced directly by the catabolism of several amino acids including serine, glycine, histidine and tryptophan, and by the recycling of methylthioadenosine from the polyamine biosynthesis pathway, as well as by the oxidation of formaldehyde.

Toxicokinetics of methanol

18. Methanol is readily absorbed by ingestion, inhalation and dermal exposure. Ingested methanol is absorbed within 30-60 minutes, depending on the presence or absence of food in the gut.

19. Methanol readily enters the total body water and is distributed to organs and tissues in direct proportion to their water content. The volume of distribution is 0.6-0.7 L/kg bw¹.

20. After uptake, most of the methanol (96.9%) is converted to carbon dioxide in the liver, with a small fraction excreted directly in the urine or by the lungs. Oral methanol is therefore subject to significant first pass metabolism before it reaches the systemic circulation.

21. 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 alcohol dehydrogenase (ADH). In non-primate mammals, the reaction is primarily mediated by a catalase-peroxidative system, but the conversion rates are similar. The oxidation of formaldehyde to formate is mediated by several enzyme systems including formaldehyde dehydrogenase.

22. Formate is then oxidised to carbon dioxide through the action of formyl-THF synthetase, whereby formic acid combines with tetrahydrofolic acid (THF) to form 10-formyl-THF which is subsequently converted to carbon dioxide by formyl-THF-dehydrogenase²². Formate oxidation to carbon dioxide is variable between species, and this variability determines sensitivity to acute methanol toxicity (the rate of formate elimination in humans and non-human primates is half of that in rats). The difference reflects the availability of folate. In both humans and monkeys, levels of THF are 60% and 16-26% of those found in rat and mouse liver respectively. Formyl-THF-dehydrogenase activity has also been reported to be lower in the livers of humans and monkeys, being 25% and 37% respectively of that measured in rat liver²³.

23. Methanol toxicity occurs at levels at which formate accumulates – that is, when the formate production exceeds formate clearance. The efficiency of the

formate clearance pathway is dependent on the availability of the folate cofactor. Using Michaelis-Menten kinetics it has been estimated that in a person with 60% body water, a dose of 210 mg/kg bw methanol would saturate the folate pathway²⁴. This calculation used data from non-human primates to estimate the maximum rates of formate oxidation and methanol metabolism, assumed uniform distribution of formate in the body water to scale the Michaelis constant (Km) parameter to a methanol dose, and did not distinguish whether or not the formate was produced endogenously. For a 70 kg adult the estimate is equivalent to 14.7 g of methanol, a dose consistent with those that have been associated with documented toxic effects.

24. The reactions involved in the oxidation of formate are summarised in the figure below taken from Kavet and Nauss, 1990²⁴.

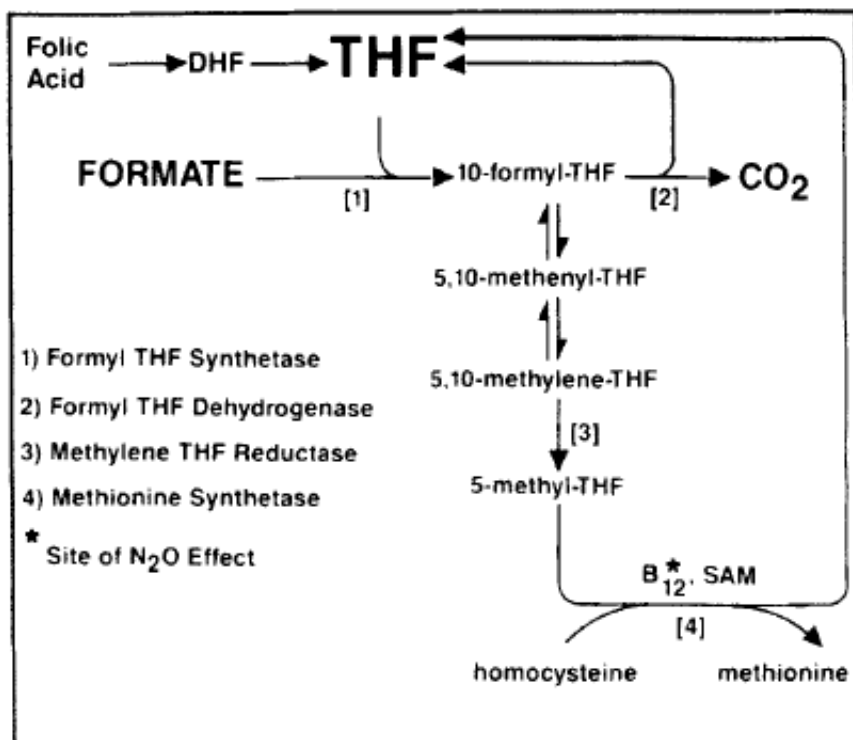


Figure 1

DHF = Dihydrofolic acid

THF = Tetrahydrofolic acid

SAM= S-adenosylmethionine

Reaction [1] requires prior activation of formate by Adenosine triphosphate (ATP)

Reaction [2] involves [¹⁰M] Formyl-THF dehydrogenase and uses NADP as a hydrogen acceptor.

Reaction [3] is catalysed by 5-methylene-THF-reductase and is essentially irreversible.

Reaction [4] is catalysed by 5-methyl-THF homocysteine methyl transferase (methionine synthetase) and requires catalytic amounts of vitamin B₁₂ and SAM.

25. Clearance of methanol is slow, particularly when compared to ethanol. Half times of 2.5-3 hours have been reported for doses of less than 0.1 g/kg methanol given to human volunteers, increasing to 24 hours or longer for doses greater than 1g/kg¹. Stegink *et al* (1981)¹⁷ reported that the methanol released from high doses of aspartame (80 mg/kg bw) cleared with half times of 2.5 to 3 h in human volunteers.

26. Methanol can be cleared from the body in two ways either as unchanged methanol or, predominantly, by metabolism to CO₂. Metabolic clearance of methanol is much faster than the excretion of unchanged methanol.

27. The clearance of unmetabolised methanol via urine and exhaled air follows first order kinetics (it is proportional to the concentration of methanol remaining in body water). When concentrations of methanol are low, its metabolism to CO₂ and subsequent clearance also follows first order kinetics, but this is a saturable process and, once saturated (which occurs at higher methanol concentrations), it follows zero order kinetics (i.e. it proceeds at a constant rate that is independent of concentration in body water). Thus at low concentrations, methanol is cleared by both routes and elimination follows first order kinetics. At medium concentrations, clearance occurs by both routes but zero order kinetics applies as metabolic clearance predominates but is saturated. At very high concentrations, the excretion of unchanged methanol becomes predominant, and the kinetics revert to first order. Expressing this in terms of blood methanol concentrations, data from monkeys indicate that methanol elimination would be first order at concentrations of 20-100 mg/L methanol, zero order at 100-3000 mg/L and first order at concentrations > 3000 mg/L^{25,26}.

Methanol and formate concentrations in blood following different types of methanol exposure

Background concentrations

28. According to the IPCS Poisons Information Monograph (PIM, 1991)²⁷, normal blood methanol concentrations are in the order of 15 mg/L (range 2-30 mg/L). However, the majority of individual studies report methanol concentrations at the lower end of this range. Urinary methanol concentrations have been reported to be up to 3 mg/L in individuals not occupationally exposed to methanol²⁸. Background concentrations of formate in blood are reported to be in the range 0 to 18 mg/L²⁷ (but are usually at the lower end of this range. Urinary formate concentrations are in the range 2-30 mg/L.

Blood concentrations associated with dietary exposure

Methanol

29. In human volunteers, consumption of either 10-15 g isolated pectin or 1 kg apples, containing approximately 10 g of natural pectin, induced a significant increase in methanol in the breath and, by inference, in the blood³. The amount of methanol generated from the pectin (0.4-1.4 g) was comparable to the total daily endogenous production of methanol (0.3-0.6 g) measured by the same group. Consumption of 1 kg apples was estimated to release 500 mg methanol. The

authors cited earlier work showing that concentrations of methanol in the breath as high as 3 ppm (reported to be equivalent to 10 mg/L in blood) were produced in human volunteers consuming 0.75 kg peaches/apples, and that ingestion of 13.3 g pectin by volunteers, three times a day over 2 days increased serum methanol to a maximum of 50 mg/L. These experiments used concomitant consumption of ethanol to inhibit methanol metabolism so that it could be measured.

30. No other data have been identified linking dietary consumption of food with changes in blood or urinary methanol and formate concentrations.

Aspartame

31. Following a single dose of 500 mg aspartame to four adult male volunteers (equivalent to 6-7.8 mg/kg bw), serum methanol concentrations increased from baseline²⁹. Concentrations were highest 45 minutes after consumption, when a mean increase of 1.06 mg/L methanol was observed. However, mean serum methanol concentrations at 30, 60 and 90 minutes after aspartame ingestion were also higher than baseline. Two hours after consumption, serum methanol concentrations had returned to baseline. The aspartame dose used was equivalent to that found in 0.83 L of diet soft drink.

32. Blood methanol concentrations were measured in 30 adult subjects given doses of 34, 100, 150 or 200 mg/kg bw aspartame¹⁷. These doses were equivalent to 0.85, 2.5, 3.75 and 5 times the ADI. Blood samples were taken at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 8 and 24 hours after dosing. Methanol concentrations were below the limit of detection (4 mg/L) at all times in the 34 mg/kg bw group. At the higher aspartame doses (100 mg/kg bw and above), blood methanol concentrations were significantly elevated in each dose group, with mean peak blood methanol concentrations and the areas under the blood methanol concentration time curve increasing in proportion to dose. Mean peak blood concentrations were 12.7, 21.4 and 25.8 mg/L in the 100, 150 and 200 mg/kg bw groups respectively, occurring about 2 hours after dosing. Blood methanol concentrations had returned to baseline 8 hours after dosing with 100 mg/kg aspartame but were still detectable in the 150 and 200 mg/kg bw dose groups. However, methanol was no longer detectable in the blood in any subjects 24 hours after treatment. Blood and urinary formate concentrations were measured in the six subjects in the 200 mg/kg bw dose group. Blood formate concentrations did not increase, but urinary formate concentrations increased significantly over pre-loading values in the samples obtained 0-4 and 4-8 h after loading, then returned to pre-loading concentrations in the 8-24 h sample. This indicated that an increase in conversion of methanol to formate occurred but that formate production did not exceed excretion (otherwise blood concentrations would have increased).

33. In a further study by the same group³⁰, one year old infants were given doses of aspartame of 34, 50 or 100 mg/kg bw. Again, methanol concentrations were below the limit of detection of 3.5 mg/L in the blood of the infants who had consumed the lowest dose but were significantly elevated in infants receiving the higher doses of aspartame, with mean peak blood methanol concentrations and the areas under the blood methanol concentration-time curve increasing in proportion to dose. When compared to adults who had received an equivalent aspartame dose, the blood methanol concentrations were similar (peaking at 30-90 minutes after

administration), as were the areas under the blood methanol concentration-time curve for the first 2.5 hours.

34. For the infants receiving the 100 mg/kg bw dose of aspartame, blood methanol concentrations were also similar to those of adults, peaking at 90 minutes after administration, but the area under the blood methanol concentration-time curve for the first 2.5 hours was significantly lower than in adults. The authors considered that the blood methanol concentrations of the infants would have returned to baseline at 8 hours. Because of limitations in the size of the sample that could be taken, blood formate concentrations were not measured. The authors noted that the absorption and metabolism of methanol from a methyl ester such as aspartame may be slower than that of free methanol, since aspartame must pass into the small intestine before hydrolysis to release methanol.

35. Six young adults consumed eight successive 8 oz (227 ml) servings of a drink at one-hour intervals³¹. The drink was either unsweetened or sweetened with 600 mg aspartame (equivalent to 8.5 mg/kg bw aspartame per serving). During the course of the experiment a total of 4.8 g aspartame was consumed, being equivalent to 80 mg/kg bw for a 60 kg individual or twice the ADI. Blood methanol concentrations remained below the limit of detection (3.5 mg/L) while blood formate concentrations were not significantly different from initial baseline concentrations following the consumption of either the aspartame-sweetened or the unsweetened beverage. Urinary formate concentrations were also not significantly different. The authors concluded that the metabolism of methanol following each dose of aspartame was sufficient to prevent either methanol or formate accumulating in the body as successive doses were consumed. Methanol was not detected in the blood or urine of 33 subjects randomly selected from a study population of 126 children and adolescents who had consumed 0.61, 0.8, 1.6, 2, and 2.4 g/day aspartame in a range of food products during a 13 week double-blind study³². The limit of detection was not reported and other details of the experiment were limited.

Blood concentrations associated with occupational exposure

36. A number of studies have been conducted in human volunteers exposed to methanol vapour at the widely adopted occupational exposure limit (OEL) of 200 ppm for periods ranging from 75 minutes to 7 hours^{33, 34, 35, 36}. These suggest that following such exposure, blood methanol concentrations increase transiently but blood formate concentrations do not increase. At the same time, both methanol and formate concentrations in urine increase, suggesting that the exposure to methanol leads to an increase in methanol metabolism and formation of formate, but that at the concentrations produced, the formate is readily metabolised and excreted as CO₂ and does not accumulate. In these studies, methanol and formate concentrations were not measured during the exposure period, so conclusions cannot be drawn on the length of exposure before concentrations become elevated.

37. In a study by Ferry *et al* (1980)³⁷ volunteers ingested small quantities of methanol at regular intervals to mimic industrial exposure. The concentration of methanol in urine correlated with that in the blood, both as concentrations increased following doses of 0.2 ml (0.16 g) every hour for six hours and as concentrations declined following a single dose of 3 ml (2.37g) of methanol. In an experiment in which small hourly doses were consumed by three subjects, urinary methanol did not

increase above 8 mg/L. Detailed data on blood methanol concentrations were not reported, but Figure 1 of the paper, suggests that blood methanol also did not exceed 8 mg/L. The authors considered that urinary formic acid concentration was too variable to be a useful marker of occupational exposure to methanol but that formic acid excretion rate (formic acid concentration corrected for creatinine concentration) in urine correlated with methanol exposure and had the potential to be used for monitoring of occupational exposure. No information was reported on adverse effects, if any, among the volunteers.

Blood concentrations associated with toxicity

38. WHO (1997)⁷ stated that, in general, blood methanol concentrations > 200 mg/L are associated with central nervous system (CNS) effects, concentrations > 500 mg/L with severe acute toxicity and concentrations > 1500-2000 mg/L with fatality in untreated patients.

39. Serum formate and methanol concentrations are not well correlated - possibly due to high inter-individual variation and the inhibition of methanol metabolism by concomitant ethanol consumption in many of the reported incidents of methanol poisoning in humans. In cases of acute poisoning, blood methanol concentrations did not correlate with degree of bicarbonate depression (an indirect indicator of acidosis) and did not predict gastrointestinal, nervous system or ocular symptoms except when extremely elevated³⁸.

40. However, as there is a lag between initial methanol exposure and the subsequent appearance of toxic symptoms, blood concentrations of methanol may be low or at background at presentation and may not relate either to the initial exposure or to the final prognosis. Jacobsen and McMartin (1986)³⁹ stated that there was no relationship between the blood concentration of methanol and the degree of toxicity in human case reports. It has been suggested that blood formate may be a better indicator of toxicity since it is the accumulation of formate that results in formate acidosis and ocular injury.

41. Excessive production of formic acid leads to metabolic acidosis and elevated formate concentrations which can reach 10-20 mM (460-920 mg/L) in severe cases. Data from case series suggest that visual dysfunction is apparent when formate concentrations exceed 200-300 mg/L, with formate concentrations of >500 mg/L at the time of hospital admission being associated with poor visual prognosis or death⁴⁰.

Modelling

42. There have been various attempts to use Physiologically-Based Pharmacokinetic (PBPK) modelling to assess and predict methanol toxicity from a given exposure.

43. The models include one developed by Ward *et al* (1997)⁴¹ to describe methanol disposition in pregnant rats and mice, which they considered agreed well with relevant data from the literature. Horton *et al* (1997)⁴² investigated the pharmacokinetics of methanol in rhesus monkeys (when administered by inhalation)

and in rats (when administered by inhalation or intra-venously). They then developed a model to simulate the *in vivo* data, which could be scaled for application to human exposures. This model was used to investigate the range of atmospheric concentrations over which kinetics in the two laboratory species were quantitatively similar to those in humans. This was reported to be up to 1200 ppm. Bouchard *et al*, (2001)⁴³ developed a biologically based pharmacokinetic model to simulate the effect of methanol inhalation, based on published kinetic data in different laboratory species and from human volunteers. Using this, they concluded that 8 hour inhalation exposures of 500-2000 ppm methanol (in the absence of physical activity) would be required to raise blood formate and urinary formic acid concentrations above background.

44. Simpler methods of estimation have been used based on the assumption that methanol would be readily absorbed and distributed to the body water. This was applied to ingestion of aspartame by Stegink and colleagues¹⁷, with the assumption that aspartame was instantly hydrolysed in the intestinal lumen and that the methanol released was rapidly absorbed and distributed to total body water (55% of body weight). The predicted maximal blood methanol concentrations were similar to those estimated by extrapolating experimentally measured blood methanol time curves (in which the first measurement was taken at one hour) back to time zero. This observation suggests that the assumptions of instant hydrolysis, rapid absorption and distribution to total body water could be useful in predicting the effects of various doses of methyl esters on blood methanol concentrations.

45. The application of modelling methods to the assessment of methanol exposure and toxicity is complicated by uncertainty about the tissue measure of methanol exposure that is most relevant to toxicity. Blood formate appears to be the most appropriate measure to assess overt toxicity, but for monitoring of occupational exposure, blood or urinary methanol may be more practical when the samples are taken soon after exposure.

Methanol toxicity

46. The vast majority of human data on methanol toxicity relate to acute or short term effects. 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)¹. The minimum dose associated with ocular toxicity is unclear but may be 10 mls (8 g or 133 mg/kg bw)⁴⁴. There is wide inter-individual variability in the toxic dose. It has been reported that the most important determinants of susceptibility to methanol toxicity are concurrent ingestion of ethanol, which reduces susceptibility, and folate status in the liver which may be associated with increased susceptibility if deficient¹.

Symptoms

Acute

47. In species that metabolise formate poorly, such as primates, acute and short term methanol toxicity is characterised by formic acidemia, metabolic acidosis, ocular toxicity, nervous system depression, blindness, coma and death. In species

which metabolise formate readily, fatal acute toxicity is usually through CNS depression. Longer term exposure to lower levels of methanol may cause various ocular effects such as blurred and misty vision.

48. In humans, the symptoms and signs of acute 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 including coma and seizures. The ocular effects develop 12-48 hours after methanol ingestion and range from mild photophobia, through misty or blurred vision to significantly reduced visual acuity or complete blindness. Visual disturbances have been reported in workers exposed to airborne methanol concentrations of 1500 mg/m³ (1200 ppm) or higher. End stage manifestations of irreversible damage such as pallor of the optical disc may appear 1-2 months after acute exposure.

49. Toxicity occurs when formate production exceeds clearance over a sustained period. Undissociated formic acid binds to, and inhibits, the mitochondrial enzyme cytochrome oxidase causing histotoxic hypoxia, and thereby inhibits retinal and optic nerve mitochondrial function and depletes retinal and optic nerve ATP⁴⁵. Depletion of ATP results in reduced activity of membrane Na-K ATPase pump, which in turn halts conduction of the action potential, damages the myelin sheath and causes loss of vision. It also leads to stasis of axoplasmic flow, which then results in intra-axonal swelling and optic disc oedema. Swelling in myelin sheaths causes compression injury to the nerve fibres, preventing further axoplasmic flow of proteins, mitochondria and neurotubules from the cell body to the fibre of the axoplasm; as the cells become deficient in these, they become more sensitive to formic acid-induced injury, which further exacerbates neuronal conduction deficits and loss of vision.

50. The selective damage to the retro laminar section of the optic nerve and retina may be caused by particularly high exposure to formic acid due to copious blood flow through the choriocapillaris (a layer of capillaries beneath the retina) and additionally through the diffusion of formic acid from the cerebral spinal fluid to the adjacent optic disc and the retrolaminar section of the optic nerve. Furthermore, optic nerve fibres and their myelin sheaths are unusually sensitive because they have fewer mitochondria and lower reserves of cytochrome oxidase than other cells due to their low metabolic requirements. Retinal dysfunction documented by visual evoked potentials occurs at lower formic acid concentrations than optic neuropathy.

51. Although the ocular toxicity appears to be a direct effect of formic acid rather than the acidosis generated by the formic acid⁴⁶, acidosis increases toxicity by enabling greater diffusion of formic acid into cells. Reduction of acidosis helps to protect vision as it increases the amount of formate which does not diffuse as readily as undissociated formic acid.

52. The neurotoxic effects of methanol are also due to inhibition of cytochrome oxidase and the Na-K ATPase pump by formic acid.

53. While the main acute toxic effects of methanol result from the accumulation of formate, it is possible that some adverse effects may be attributable to un-metabolised methanol. However, no data could be found to support this.

Chronic toxicity

54. A limited number of case reports suggest that extended exposure to methanol may cause effects qualitatively similar to those arising from acute exposure including, in some cases, central nervous system (CNS) and visual disorders. For example, in workers regularly operating spirit duplicators (which used a 99% methanol duplicator fluid), symptoms such as dizziness, headaches, nausea and visual disturbances were reported⁴⁷. 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.

52. A widely used occupational exposure limit (OEL) for methanol is a time weighted average of 200 ppm for 8 hours exposure. This is designed to protect workers from any methanol-induced formic acid metabolic acidosis and ocular and nervous system toxicity. A concentration of 200 ppm methanol is equivalent to 260 mg/m³; assuming that an average human breathes 22 m³ air in a 24 hour period (consisting of 8 hours rest and 16 hours light non-occupational activity)⁴⁸ and hence $22/24 \times 8 = 7.3$ m³ air in 8 hours, it can be estimated that $7.3 \times 260 = 1900$ mg methanol would be inhaled during that time period. This is equivalent to 31.7 mg/kg bw methanol in a 60 kg adult. However, the activity that would occur in an occupational situation increases inhalation and therefore methanol exposure. For example, Lee *et al*, (1992)³⁷ reported that at this level of exposure, light activity increased methanol inhalation in volunteers (as assessed by the measurement of pulmonary respiration) 1.8 fold compared to the level of inhalation when resting, and thus the OEL is likely to be conservative for non-occupational exposure.

Reproductive effects- methanol

53. There are few data on adverse reproductive effects in humans. Case report data suggest that methanol can pass from the mother to the fetus and result in toxicity^{49, 50, 51}. Maternal occupational exposure to methanol was not associated with an increase in the occurrence of cleft lip and palate⁵²

54. Likewise, there are few data on the reproductive effects of methanol in experimental animals. In rodents, very high doses of methanol (generally > 2 g/kg bw) are associated with a range of teratogenic effects when administered by inhalation^{53, 54, 55} or orally⁵⁶.

55. In the only reported primate study⁵⁷, groups of 11-12 Macaque monkeys were exposed to 0, 200, 600 or 1800 ppm methanol vapour for 2.5 h/day for 7 days a week prior to breeding and throughout pregnancy (estimated doses of 0, 11, 33 and 98 mg/kg bw/day). In the maternal animals, methanol exposure did not affect menstrual cycles, number of matings to conception or conception rate. The mean length of pregnancy was significantly reduced in treated animals (6-8 days less than in the controls), but the reduction was not dose-related. The authors suggested that a modest, but biologically significant, effect on the fetal hypothalamic-pituitary-adrenal axis might have occurred, resulting in reduction in the length of pregnancy. In addition, although not statistically significant, five methanol treated females (in different dose groups) were Caesarean- sectioned due to pregnancy complications.

No methanol-related effects were apparent on the birth weight or health of the offspring.

Reproductive effects - aspartame

56. In a prospective epidemiological study by Halldorsson *et al* (2010)⁵⁸, consumption of artificially sweetened soft drinks was associated with an increased risk of pre-term delivery. The individual sweeteners used were not specified, but were likely to be aspartame or acesulfame-K. Without independent replication, the interpretation of this finding is uncertain.

57. Studies of aspartame¹ in rodents^{59, 60, 61, 62} did not indicate any adverse effects at concentrations of 4% in the diet (3500- 600 mg/kg depending on the stage of the study). Aspartame has not been studied for reproductive effects in non-human primates.

Sensitive sub groups

58. Although there is no clear evidence of methanol causing specific adverse reproductive effects in humans or non-human primates, it is possible that pregnant women might be more sensitive to methanol toxicity because their folate requirements are increased during pregnancy and they can readily become deficient. Folate is needed for one-carbon transfer reactions including those required for DNA synthesis in the growing fetus and placenta and for increased production of maternal red blood cells⁶³. Red cell (rather than serum folate), indicates the status of liver folate stores, and it has been reported that during pregnancy, red cell folate concentrations decline. For example, Chanarin *et al* (1968)⁶⁴ found that red cell folate decreased from 6.1 mg/L at 15 weeks to 4.2 mg/L at 38 weeks. The decline can be prevented by supplementation with folic acid. Similarly, Milman *et al* (2006)⁶⁵ reported that median red cell folate concentrations declined from 0.84 µmol/L at 18 weeks gestation to 0.75, 0.65 and 0.55 µmol/L at 32 weeks, 39 weeks and postpartum respectively. This is equivalent to 0.37, 0.33, 0.29 and 0.24 mg/L. It has been suggested that red cell folate concentrations of <0.33 µmol/L could be considered deficient, 0.33-0.42 µmol/L intermediate and 0.42-1.46 µmol/L normal⁶⁶.

59. It is well established that manipulation of folate can increase or decrease sensitivity to methanol toxicity in experimental animals, and folate-depleted rodents have been used as a model for methanol toxicity in primates. There are fewer data on how methanol toxicity in primates might be affected by folate deficiency. However, in a study by Dorman *et al* (1994)⁶⁷, cynomolgus monkeys were exposed to 10, 45, 200 or 900 ppm ¹⁴C-labelled methanol for 2 hours and then placed on a folate deficient diet for approximately 6 weeks until red cell folate concentrations decreased to a level consistent with moderate deficiency (29-107 mg/L). The animals were then re-exposed to 900 ppm methanol for 2 hours. End of exposure blood

¹ The data on aspartame submitted as part of the initial approval process have not been considered in this review.

methanol concentrations \pm standard deviations (SD) were 0.65 ± 0.3 , 3.0 ± 0.8 , 21 ± 16 , 106 ± 84 and $211 \pm 71 \mu\text{M}$ for the 10, 45, 200, 900 and 900FD (folate deficient) exposures (equivalent to 0.021 ± 0.009 , 0.096 ± 0.026 , 0.67 ± 0.51 , 3.39 ± 2.7 and $6.8 \pm 2.3 \text{ mg/L}$) while peak blood formate concentrations were 0.07 ± 0.02 , 0.25 ± 0.09 , 2.3 ± 2.9 , 2.8 ± 1.7 and $9.5 \pm 4.7 \mu\text{M}$ (0.0032 ± 0.0009 , 0.012 ± 0.004 , 0.1 ± 0.13 , 0.13 ± 0.08 , $0.44 \pm 0.22 \text{ mg/L}$). Although higher in the folate-deficient monkeys, the blood concentration of [^{14}C] methanol derived formate was 10-1000 times lower than the endogenous blood formate concentration (0.1-0.2mM or 4.6-9.2 mg/L) for monkeys. The authors concluded that low folate concentrations would not result in elevated blood formate concentrations in humans under short-term exposure conditions.

Ethanol/methanol interaction

60. Since ethanol is preferentially metabolised by ADH, it is able to block the metabolism of methanol. This is used in the treatment of methanol poisoning, in which ethanol is infused to achieve a constant blood concentration of 1000-1500 mg/L ethanol⁴⁵. Methanol concentrations gradually decline through non-metabolic clearance and when they reach the concentration at which the subsequent formate production would not be harmful, the ethanol is withdrawn and methanol metabolism resumes. The quantity of ethanol used is significantly higher than the amount of ethanol produced endogenously or from dietary sources (excluding alcoholic beverages). As noted earlier, the interaction between methanol and ethanol is also used experimentally to allow endogenous methanol to be measured – for example, see Lindinger *et al* (1997)³ in which volunteers were given two 75 g doses of a 40:60 % ethanol; water mix to block methanol metabolism for 5 ½ hours.

Summary and conclusions

61. Methanol is produced endogenously at a rate of approximately 300-600 mg/day.

62. External exposure to methanol arises from a variety of sources. Dietary methanol has been estimated to be up to 1g/day, the total being likely to depend on how much fruit, vegetables and their juices are consumed. Consumption of aspartame at the ADI (40 mg/kg bw) would result in exposure to 240 mg methanol in a 60 kg adult, but as estimated exposures to aspartame are below the ADI, methanol exposure from this source is likely to be less than 240 mg in practice.

63. Occupational exposure to methanol vapour at the OEL of 200 ppm would result in exposure to approximately 1.9 g of methanol over an 8 hour period. This exposure level would not be expected to cause toxicity.

64. The minimum lethal dose of methanol is 0.3-1g/kg bw (WHO, 1997) equivalent to 18–60 g in a 60 kg adult. The minimum dose causing ocular toxicity is less certain but may be as low as 10 ml (8 g).

65. Methanol toxicity occurs when formate production exceeds formate clearance over a sustained period, allowing the accumulation of formic acid and the development of formic acidosis. Undissociated formic acid binds to cytochrome oxidase resulting in histiocytic hypoxia, inhibition of mitochondrial function and depletion of ATP. The rich blood supply to the optic nerve and the low reserves of cytochrome oxidase in the optic nerve and retina underlie the ocular toxicity associated with methanol poisoning. Metabolic acidosis alone does not account for methanol toxicity, but it increases the formation of undissociated formic acid, thereby enhancing toxicity. Documented methanol toxicity in humans has generally resulted from acute, high level exposures.

66. There are fewer data on the chronic effects of methanol exposure. Symptoms such as headache, dizziness, gastrointestinal upset and visual disturbance have been reported after occupational exposure to methanol vapour at levels in excess of the OEL, but not at levels below the OEL. Volunteer studies indicate that inhalation exposure at regulatory limits results in transient increases in blood and urinary methanol and urinary formate concentrations but not in blood concentrations of formate, suggesting that the methanol is being metabolised and excreted but formate is not accumulating.

67. Volunteer studies indicate that exposure to aspartame at levels approximating the ADI, does not result in measurable changes in blood or urinary methanol or formate concentrations. Higher exposure results in transient increases in blood and urinary methanol and urinary formate concentrations but not in blood formate, suggesting that as with occupational exposure at the OEL, the methanol is being metabolised and excreted but formate is not accumulating.

68. Given that:

a) Intake of methanol from the diet, including from currently permitted levels of aspartame, is below that which would occur from occupational exposure at the OEL;

b) There is no increase in blood formate either after experimental inhalational exposure at the OEL, or after oral dosing with aspartame at doses well in excess of its ADI; and

c) No adverse health effects have been reported from long-term occupational exposures to methanol at levels below the OEL;

we conclude that exposure to methanol at the levels found in the diet, both naturally occurring and from currently permitted levels of aspartame, would not be expected to result in adverse effects.

69. Subjects with low folate status such as pregnant women may be more sensitive to methanol toxicity. It is uncertain whether this could occur at levels of methanol exposure comparable to those in the diet, but the limited data that are available for non-human primates suggest that this is unlikely.

68. The main uncertainties in this risk assessment stem from the limited direct evidence on effects of chronic methanol exposure. However, given the relatively high rate at which the compound is produced endogenously, and the large body of

evidence indicating that consumption of fruit and vegetables is good for health, we consider that further research on dietary methanol should not be a high priority.

COT statement 2011/02
March 2011

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ABBREVIATIONS

ADH	Alcohol Dehydrogenase
ADI	Acceptable Daily Intake
ATP	Adenosine Triphosphate
BW	Body Weight
C¹⁴	Carbon 14
CNS	Central Nervous System
CO₂	Carbon Dioxide
COT	Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment.
DHF	Dihydrofolic acid
DINFO	Daily intake via natural Food Occurrence
DNA	Deoxyribonucleic acid
EC	European Commission
EPA	Environmental Protection Agency
EU	European Union
FD	Folate deficient
FSA	Food Standards Agency
g	gram
h	hour
IPCS	Interational Programme on Chemical Safety
Kg	Kilogram
L	Litre
m	Metre
µmol	Micromoles
mg	Milligram
ml	millilitre
mmol	Millimoles
mM	Millimolar
NADP	Nicotinamide Adenine Dinucleotide Phosphate
Na-K	Sodium-potassium
NIOSH	National Institute for Occupational Health and Safety
OEL	Occupational Exposure Limit
PBPK	Physiologically Based Pharmacokinetic
PCM	Protein carboxymethylase
PIM	Poisons Information Monograph
PPM	Parts per million
SAM	S-adenosylmethionine
SD	Standard deviation
THF	Tetrahydrofolic Acid
UK	United Kingdom
US	United States
VD	Volume of Distribution
vol	Volume
%v/v	percentage volume/volume
WHO	World Health Organisation