TOX/2010/30

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

CHRONIC TOXICITY OF METHANOL – SUMMARY OF DATA CONSIDERED

Introduction

1. Methanol toxicity is well characterised as an acute effect occurring at high levels of exposure, but less is known about chronic effects at lower levels of exposure. The COT has been conducting an ongoing review of chronic oral methanol exposure in the light of consumer concerns that methanol arising from the breakdown of the sweetener aspartame could be harmful. A range of data has now been considered by the committee. This has been summarised below and will form the basis of the eventual statement.

2. Members are asked to comments on the conclusions set out in paragraph 54 and to comment on the general structure and content of the summary below.

Summary of methanol data

3. Methanol (CH_3OH) is a colourless, volatile liquid with a mild alcoholic odour when pure. It is miscible with water and organic solvents such as acetone (WHO,1997).

4. Exposure to methanol may occur through industrial exposure and consumption of counterfeit or illegally distilled spirits. Methanol also occurs in the diet and is produced endogenously.

Dietary methanol

5. 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 is released by digestion of the methoxy groups on polysaccharides such as pectin. In fruit juices, the methanol content ranges from 1- 640 mg/L with an average of 140 mg/L (WHO, 1997). Consumption of 10-15 g isolated pectin or the natural pectin in 1kg apples induces a significant increase in methanol in the breath and, by inference, in the blood (Lindinger *et al*, 1997). It was estimated that 0.4-1.4 g methanol was released from pectin, comparable to total daily endogenous production (0.3-0.6g) and that consumption of 1kg apples could release 500 mg methanol. 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.

6. Concentrations of methanol as high as 3 ppm in the breath (reported to be equivalent to 10 mg/L in blood) are produced in humans consuming 0.75 kg peaches/apples (cited Lindinger *et al*, 1997). Previous work also cited by these

authors showed 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 ethanol to inhibit methanol metabolism so that it could be measured.

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, but mean and 95th percentile intakes of 10.7 and 33 mg/day have been reported in US consumers using Daily intake via natural Food Occurrence (DINFO) analysis (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. In contrast, Monte (1984) proposed that methanol exposure from natural sources is much less than 10 mg/day. This is based on an estimate from an EPA document (Cleland and Kingsbury, 1977) and no further details are provided.

8. 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, pectin being broken down during production leading to the release of methanol. Since 2008 there has been an EU limit on methanol in vodka of 10 grams per hectolitre of 100 % vol. alcohol (which equates to 100 mg methanol per litre of alcohol or 30 mgs in 1L of 30% spirits). Data from incidents show that some illegally distilled or counterfeit alcoholic drinks contain much higher levels of methanol.

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 gut and releases a maximum of 10% methanol by weight. The Acceptable Daily Intake (ADI) for aspartame is 40 mg/kg 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. Thus, the amount of methanol potentially 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 completely sweetened with aspartame (WHO, 1997).

10. Surveys suggest that in the UK, the maximum exposure to aspartame is likely to be much lower. For example, the estimated exposure of high level (97.5%) child consumers of diet soft drinks was 12 mg/kg bw aspartame as a result of soft drink consumption (FSA, 2003); though this does not take into account other sources of exposure. The maximum permitted level for aspartame in diet soft drinks is 600 mg/L (EC, 1995). 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).

11. 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 supplements (Altaweel *et al*, 2009). However, at present, this would not be a permitted source of calcium for food supplements in the EU.

Endogenous methanol

12. Methanol is present in blood, urine, saliva and breast milk (WHO, 1997).

13. A "typical human" produces 300-600 mg methanol/day as a product of intermediary metabolism (cited Lindinger *et al*, 1997). This arises from the action of protein carboxylmethylase, a methyltransferase enzyme system first reported by Axelrod and Daly (1965) which metabolises S-adenosylmethionine to methanol and S-adenosylhomocysteine. This system is highly localised in the pituitaries of a number of mammalian species including man. Later work (Kim, 1973, Morin and Liss, 1973 discussed Stegink *et al*, 1981) demonstrated that this enzyme methylates the free carboxyl groups of proteins. Methanol is formed as the end product of this reaction through the action of protein methylesterases.

14. Majchrowicz and Mendelson, (1971) reported that mean blood methanol increased from 20 mg/L following an alcohol-free week, to 270 mg/L in alcoholic subjects allowed to drink freely for 11 days, at which time blood ethanol levels were between 1500 and 4500 mg/L. The authors did not attempt to calculate how much methanol was being produced, but it could be estimated that blood levels increased by 250/11 = 23 mg/L/day; assuming total body water of 49 L, this would imply exposure to 1.2 g/day methanol from endogenous production and external exposure.

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

Endogenous formic acid

16. Formic acid is produced by the catabolism of several amino acids including serine, glycine, histidine and tryptophan and by the recycling of methylthioadenosine from the polyamine biosynthesis pathway. Metabolic processes such as *O*- and *N* – demethylation reactions of endogenous and exogenous molecules generate formaldehyde.

17. Formaldehyde in the body has been estimated to be 2.6 mg/kg bw and can be assumed to be present in all aqueous body fluids due to its high aqueous solubility (Dhareshwar and Stella, 2007).

18. The value of 0.13-1.03 g/ day methanol exposure estimated by Dhareshwar and Stella (2007) was used to then calculate formaldehyde exposure from methanol to be 0.11-0.96 g/day, but this appeared to represent only a small fraction of the formaldehyde in systemic circulation. Using a volume of distribution (Vd) of 49 L (approximately total body water) and an equilibrium concentration of 2.6 mg/L,

formaldehyde content in the body can be estimated to be 122.5 mg (Dhareshwar and Stella, 2007). To maintain the equilibrium concentration of 2.6 mg/L, the daily turnover of formaldehyde would be 31-59 g/day (41 mg/min x 60 min x 24h), meaning that external sources of formaldehyde account for only 1-2% of the total daily turnover.

Pharmacokinetics of methanol

19. 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.

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

21. 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.

22. 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 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.

23. 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-dehydrogenase (Cruzan, 2009). Formate oxidation to carbon dioxide is variable between species, which determines sensitivity to methanol toxicity (the rate in humans and non-human primates is half that in rats). This variability is due to the availability of folate. In humans and monkeys, the levels of THF are 60% and 16-26% of those found in rat and mouse liver respectively. Formyl-THF-dehydrogenase activity was also reduced in humans and monkeys, being 25% and 37% respectively of the levels found in rat liver (Johlin *et al*, 1987). The reaction is summarised in the figure below adapted from Kavet and Nauss, 1990.

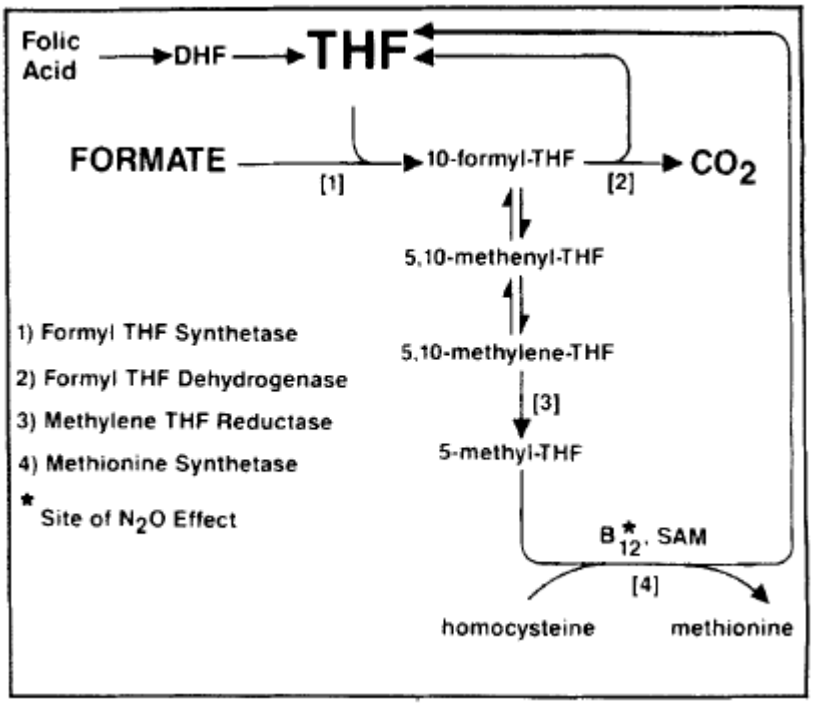

Figure 1.

DHF = Dihydrofolic acid

THF = Tetrahydrofolic acid

SAM= *S*-adenosylmethionine

Reaction [1] requires prior activation of formate by ATP

Reaction [2] involves [^{10}N] 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.

24. 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.

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 but increasing to 24 hours or more for doses greater than 1g/kg (WHO, 1997). Stegink *et al* (1981) reported that the methanol released from

high doses of aspartame (80 mg/kg bw) cleared with half times of 2.5 to 3h in human volunteers.

26. The clearance of unmetabolised methanol through urine and exhaled air follows first order kinetics (it is dependent on dose). However, methanol metabolism to CO_2 and its subsequent clearance is a saturable process, and follows zero order kinetics (it proceeds at constant rate and is independent of dose). Thus at low levels of methanol exposure, methanol is cleared by both routes but first order clearance predominates. At medium levels, clearance by both routes occurs but zero order predominates. At very high levels, the metabolic process is saturated, formate starts to accumulate, but unchanged methanol is still excreted and overall first order kinetics dominate. Expressing, this in terms of blood methanol levels, data from monkeys indicates that in terms of blood methanol levels, elimination would be first order at levels of 20-100 mg/L methanol, zero order at 100-3000 mg/L and first order at levels > 3000 mg/L (Jacobsen *et al*, 1988, Tephly *et al*, 1991).

Methanol toxicity

27. The vast majority of human data on methanol toxicity 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. It has been reported that the most important determinants of susceptibility to methanol toxicity are concurrent ingestion of ethanol and folate status in the liver (WHO, 1997).

Symptoms

Acute

28. 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 such as blurred and misty vision. In species which metabolise formate readily, central nervous system (CNS) depression is usually the cause of death.

29. 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/m^3 (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.

30. 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.

31. Numerous case reports of poisoning incidents have been published including, Bennett *et al*, 1953, Roe, 1982, and Naraqi *et al*, 1979.

Chronic/occupational toxicity

32. 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 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 these data, WHO (1997) concluded that adverse effects may become apparent where the threshold limit value of 260 mg/m^3 (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.

33. A widely used occupational exposure limit for methanol is a time weighted average of 200 ppm for 8 hours exposure. This is designed to protect workers from any of the effects of methanol-induced formic acid metabolic acidosis and ocular and nervous system toxicity. A concentration of 200 ppm methanol is equivalent to 260 mg/m^3; assuming that an average human breathes 22 m^3 air in a 24 hour period (WHO, 1999) and hence 22/24 x 8 = 7.3 m^3 air in 8 hours, it can be estimated that 7.3 x 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

Sensitive sub groups

34. Since folate is a necessary co-factor for the metabolism of methanol, it is possible that pregnant women might be*...[To be completed following committee discussions.]*

Blood levels associated with toxicity

35. Exposure to methanol can increase the levels of methanol and formate in both blood and urine.

36. Normal blood levels of methanol are generally in the range 0.3-2.5 mg/L, though higher levels have been reported (30 mg/L). WHO (1997) stated that, in general, blood methanol levels > 200 mg/L are associated with CNS effects, levels > 500 mg/L with severe acute toxicity and initial levels > 1500-2000 mg/L with fatalities

in untreated patients. However, while blood methanol levels > 500 mg/L are associated with severe acute clinical signs of toxicity, formate concentrations may give a better indication of potential toxicity, since the accumulation of formate results in formate acidosis and ocular injury.

37. Serum formate and methanol levels are not well correlated and while an inverse relationship might be expected, this is not observed - possibly due to high inter-individual variation and the inhibition of methanol metabolism by concomitant ethanol consumption in many of the reported incidents. Blood methanol levels did not correlate with degree of bicarbonate depression and did not predict gastrointestinal, nervous system or ocular symptoms except when extremely elevated (Swartz *et al*, 1981). Jacobsen and McMartin (1986) stated that there was no relationship between the blood concentration of methanol and the degree of toxicity.

38. However, as there is a lag between initial methanol exposure and the appearance of toxic symptoms the blood levels of methanol may be low or absent at presentation and may not relate to the initial exposure or final prognosis. It has been suggested that blood formate may be a more appropriate measure of toxicity.

39. Hovda *et al*, (2005) cited an upper reference limit for serum formate of 20 mg/L (0.4mmol/L), without specifying the source of this value. Excessive production of formic acid leads to metabolic acidosis and elevated formate levels 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 admission formate concentrations of >500 mg/L being associated with poor visual prognosis or death (Hantson *et al*, 2005).

Blood levels associated with occupational exposure

40. A number of studies have been conducted in human volunteers exposed to methanol vapour at the widely used occupational limit of 200 ppm (Cook *et al*, 1991; Lee *et al*, 1992; Franzblau *et al*, 1992; Franzblau *et al*, 1993)**.** These suggest that following exposure, blood methanol levels increased transiently but blood formate levels did not increase, and both urinary methanol and formate increased suggesting that the increased exposure to methanol resulted in an increase in metabolism and formate formation but the formate was readily metabolised and excreted as CO_2 and did not accumulate.

41. In a study by Ferry *et al* (1980) volunteers ingested small quantities of methanol at regular intervals to mimic industrial exposure. The level of methanol in urine reflected that in the blood, both as levels increased following doses of 0.2 ml (0.16 g) every hour for six hours) or as levels declined following a single dose of 3 ml (2.37g) of methanol. In an experiment where small hourly doses were consumed by 3 subjects, urinary methanol did not increase above 8 mg/L. From the figures provided in the paper, this also appears to apply to blood methanol levels but detailed data are not provided. Urinary formic acid was considered to be too variable to be a useful marker of occupational exposure to methanol, but formic acid excretion rate (formic acid/creatinine) reflected methanol uptake and could be used for monitoring, though measurement of creatinine represented an additional

analytical burden. No details are provided on adverse effects, if any, among the volunteers.

Blood levels associated with diet

Aspartame

42. Following a single dose of 500 mg aspartame to four adult male volunteers (equivalent to 6-7.8mg/kg bw) (Davoli *et al*, 1986), serum methanol levels increased over baseline; this was highest 45 minutes after consumption, where a mean increase of 1.06 mg/L methanol was measured, but mean serum methanol levels at 30, 60 and 90 minutes after aspartame ingestion were also higher than baseline. Two hours after consumption serum methanol levels had returned to baseline. The aspartame dose used was equivalent to that found in 0.833 L diet soft drink.

43. Blood methanol levels were measured in 30 adult subjects given doses of 34, 100, 150 and 200 mg/kg bw aspartame (Stegink *et al*, 1981). These doses are 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) in the 34 mg/kg bw group. At the higher doses (100 mg/kg bw and above), blood methanol levels were significantly elevated in each dose group, with mean peak blood methanol levels and the areas under the blood methanol concentration time curve increasing proportionally. Mean peak blood levels 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 levels returned to baseline 8 hours after dosing with 100 mg/kg aspartame but were still detectable in the top 2 doses at that time point, however, blood methanol was not detected 24 hours after treatment. Blood and urinary formate levels were measured in subjects in the 200 mg/kg bw dose group, but no significant increases in levels were apparent. Blood formate levels did not increase, but urinary formate was increased over pre-loading values in the samples obtained 0-4 and 4-8h after loading, but returned to pre-loading values in the 8-24h sample. This indicated that an increase in conversion of methanol to formate was occurring but that formate synthesis did not exceed excretion as blood levels did not increase.

44. In a further study by the same group (Stegink *et al*, 1983) one year old infants were given doses of aspartame of 34, 50 or 100 mg/kg bw. Again, blood methanol concentrations were lower than the limit of detection 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 levels 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 levels were comparable peaking at 30-90 minutes after administration, as were the areas under the blood methanol concentration-time curve for the first 2.5 hours.

45. For the infants receiving 100 mg/kg dose of aspartame, blood methanol levels were also comparable 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 higher in adults. The authors considered that the blood methanol concentrations of the infants would have returned to baseline at 8 hours. Blood formate levels were not measured due to limitations of the sample size. 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.

46. Six young adults consumed eight successive 8 oz (227 mL) servings of beverage that was either unsweetened or sweetened with 600 mg aspartame at one-hour intervals (equivalent to 8.5 mg/kg bw aspartame per serving) (Stegink *et al*, 1989). 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 levels were below the limit of detection while blood formate levels were not significantly different following the consumption of aspartame sweetened or unsweetened beverage. Urinary formate was 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 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 a 13 week double-blind study (Frey, 1976). Urinary and blood methanol levels were measured in a proportion of the subjects from each age group and treatment group. Limited experimental detail is provided.

Ethanol/methanol interaction

47. 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 where ethanol is infused at a constant level, methanol levels gradually decline through non metabolic clearance and when they reach the level at which the subsequent formate production would not be harmful, the ethanol is withdrawn and methanol metabolism resumes. It is also used experimentally to allow endogenous methanol to be measured. The level used is significantly higher than the levels of ethanol produced endogenously or from dietary sources (excluding alcoholic beverages). The recommended blood ethanol concentration for treatment of methanol poisoning is 22 mmol/L (1g/L), however this is considered to be based on clinical assumptions made without confirming blood ethanol determinations (Jacobsen and McMartin, 1986). This phenomenon is also used experimentally, to allow the measurement of endogenous methanol production, for example, see Lindinger *et al* 1997 where volunteers were given two 75 g doses of ethanol to block methanol metabolism for 5 ½ hours.

Modelling

48. There have been various attempts to use PBPK modelling to assess and predict methanol toxicity from a given exposure.

49. As part of the study by Stegink and colleagues (1981) (see paragraph 43) the authors attempted to predict the maximal blood methanol concentration that would be produced by each aspartame dose. It was assumed 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 levels were 18.1, 27.2, and 36.3 mg/L at 100, 150 and 200 mg aspartame/kg bw respectively. The levels actually measured were lower than these being 12.7 ± 4.8, 21.4 ± 3.5 and 25.8 ± 7.8 mg/L respectively. However, when the blood methanol time curves were extrapolated to zero time, the blood methanol concentrations corresponding to the intercept (13.5, 24.5, and 36 mg/L) were close to the calculated values suggesting the assumptions could be useful in predicting the effects of various doses of methyl esters on blood methanol concentrations. This method predicted a blood methanol value of 6.2 mg/L for the 34 mg/kg bw dose of aspartame used in the first part of the study, but assuming that the actual value would be 70% of that predicted (as above) this would be 4.3 mg/L and at the limit of detection for the assay, in the actual study changes in blood methanol were not detected. Given the possible contribution of naturally occurring methanol from orange juice (mean reported value 140 mg/L) a small component of the methanol dose (70 mg, approximately 1mg/kg bw) may not have been from the aspartame.

50. Other models have been developed. These include Ward *et al* (1997), and Bouchard *et al*, (2001).

51. The application of modelling methods to the assessment of methanol exposure and toxicity is complicated by the uncertainty as to the appropriate tissue measure of exposure. Blood formate appears to be the most appropriate measure to assess toxicity but for monitoring of occupational exposure blood or urinary methanol may be more appropriate if the samples are taken close to the exposure. Methanol metabolism is also subject to significant individual variability.

Dose response relationship

52. Methanol is produced endogenously at a rate of approximately 300 mg-1.2 g/day. External exposure to methanol arises from a variety of sources. Dietary methanol production has been estimated to be approximately 1g/day and consumption of aspartame at the ADI (40 mg/kg bw) would result in exposure to 240 mg methanol in a 60 kg adult. Occupational exposure at the maximum permitted level of 200 ppm would result in exposure to approximately 1.9 g of methanol over an 8 hour period. None of these exposures is associated with toxicity. Volunteer studies suggest that exposures of this order result in a transient increase in blood and urinary methanol, and while blood formate is not increased, urinary formate may be, suggesting that increased exposure to methanol and formate has occurred but formate has not begun to accumulate.

53. Methanol toxicity occurs at levels at which formate begins to accumulate, that is, when the metabolic pathway is saturated and formate production exceeds formate clearance . The efficiency of the pathway is dependent on the availability of the folate cofactor. Using Michaelis menten kinetics it has been estimated that a dose of 210 mg/kg bw methanol would saturate this pathway. For a 70 kg adult this is equivalent to 14.7 g of methanol a dose consistent with the onset of documented toxic effects.

Summary

[*To be completed after committee discussions*]

Questions for the Committee

54. Members are asked to consider the following draft conclusions.

a). Methanol toxicity occurs when formate production exceeds formate clearance over a sustained period.

b). 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. The available data suggest that permitted levels of occupational exposure would also not be of concern.

c). Subjects with low folate status may be sensitive to methanol toxicity but this is unlikely to be relevant at dietary exposure levels.

55. Members are asked to make general comments on the structure and information included in the summary and whether this is an appropriate basis for a statement.

Secretariat

October 2010

REFERENCES

Altaweel, MM, Hanzlik, RP, Ver Hoeve (2009). Ocular and systematic Evaluation of Calcium Formate as a Dietary Supplement. *J. Ocul. Pharmacol. Ther.*, 25, 223-230.

Axelrod, J. and Daly, J. (1965). Pituitary Gland: Enzymic formation of methanol from S-adenosylmethionine. Science, 150 (698) 892-3.

Bennett, I.L., Cary, F.H., Mitchell, G.L., *et al*, (1953) Acute Methyl Alcohol Poisoning: A Review Based on Experiences in an Outbreak of 323 Cases. Medicine, 32, 431-463.

Bouchard, M., Brunet, R.C.,Droz, P-O., *et al* (2001). A Biologically Based Dynamic Model for Predicting the Disposition of Methanol and its Metabolites in Animals and Humans. Toxicol Sci, 64169-184.

Cleland, J.G., Kingsbury, G.L.,(1977) Multimedia Environmental Goals for Environmental Assessment. US Environmental Protection Agency E-28, November 1977.

Cook, M.R., Bergman, F.J., Cohen, H.D., *et al* (1991). Effects of Methanol Vapor on Human Neurobehavioral Measures. Res Rep Health Eff Inst, 42, 1-45.

Cruzan G (2009). Assessment of the Cancer Potential of Methanol. *Critical Reviews in Toxicology*, 39, 347-363

Davoli, E., Cappellini, L., Airoldi, R. *et al* (1986). Serum Methanol Concentrations in Rats and Men after a Single Dose of Aspartame. *Food Chemical Toxicology*, 24, 187-189.

Dhareshwar, S.S. and Stella, V.J. (2007). Your Prodrug Releases Formaldehyde: Should you be Concerned? No! *Journal of Pharmaceutical Sciences*, 97, 4184-4193.

(EC, 1995). European Parliament and Council Directive 94/35/EC of 30 June 1994 on sweeteners for use in foodstuffs (as amended) or for the UK "The **Sweeteners** in **Food** Regulations 1995" as amended.

(EC, 2008). Regulation (EC) No 110/2008 of the European Parliament and of the Council of 15 January 2008 on the definition, description, presentation, labelling and the protection of geographical indications of spirit drinks and repealing Council Regulation (EEC) No 1576/89. *Off. J. Europ. Union.* 2008;**L39**:16–54.

Ferry, D.G., Temple, W.A., McQueen, E.G. (1980). Methanol Monitoring. Comparison of Urinary Methanol Concentration with Formic Acid Excretion Rate as a Measure of Occupational Exposure. Int. Arch. Occup. Environ. Health 47, 155-163.

Franzblau, A., Levine, S.P., Schreck, R.M., *et al*, (1992). Use of Urinary Formic Acid as a Biologic Exposure Index of Methanol Exposure. Appl Occup Environ Hygiene, 7, 467-471.

Franzblau, A., Lee, E.W., Schreck, R.M., *et al*, (1993). Absence of Formic Acid Accumulation in Urine Following Five Days of Methanol Exposure. Appl Occup Environ Hygiene, 8, 883-888.

Frederick, I.J., Schulte, P.A., Apol, A. (1984). Investigation and Control of Occupational Hazards Associated with the Use of Spirit Duplicators. J Occup Environ Hyg, 45, 51-55.

Frey, G.H. (1976). Use of Aspartame by Apparently Healthy Children and Adolescents. *Journal of Toxicology and Environmental Health*, 2, 401-415.

FSA (2003). No. 36/03 Month 2003
Diary Survey of the Intake of Intense Sweeteners by Young Children from Soft Drinks.
http://www.food.gov.uk/science/surveillance/fsis2003/fsis-200336softdrink

Hantson, P., Haufroid, V., Wallemacq P. (2005). Formate Kinetics in Methanol Poisoning. *Human and Experimental Toxicology*, 24, 55-59.

Hovda, K.E., Urdal, P., Jacobsen, D. (2005a). Increased Serum Formate in the Diagnosis of Methanol Poisoning. *Journal of Analytical Toxicology*, 29, 586-588.

Jacobsen, D., and McMartin, K.E. (1986). Methanol and Ethylene Glycol Poisonings: Mechanism of Toxicity, clinical Course, Diagnosis and Treatment . *Medical Toxicology*, 1, 309-334.

Jacobsen, D. Webb, R., Collins, T.D., *et al* (1988). Methanol and Formate Kinetics in Late Diagnosed. Methanol Intoxication. *Medical Toxicology*, 3, 418-423.

Johlin FC, Fortman CS, Nghiem DD and Tephly TR (1987). Studies on the role of folic acid and folate-dependent enzymes in human methanol poisoning. *Molecular Pharmacology*, **31**, 557-561.

Kavet, R., Nauss, K.M., (1990). The Toxicity of Inhaled Methanol Vapors. CRC Crit Rev Toxicol, 21 21-50.

Kim, S. (1973). Purification and Properties of Protein Methylase II. *Arch. Bioche. Biophys*, 157, 476-484 [awaiting original]

Lee, E.W., Terzo, T.S., D'Arcy, J.B. *et al*, (1992). Lack of Blood Formate Accumulation in Humans Following Exposure to Methanol Vapor at the Current Permissible Exposure Limit of 200 ppm. Am. Ind. Hyg. Assoc. J., 53, 99-104.

Liesivuori, J and Savolainen, H (1991). Methanol and Formic Acid Toxicity: Biochemical Mechanisms. *Pharmacology and Toxicology,* 69, 157-163.

Lindinger, W., Taucher, J., Jordan, A. *et al* (1997). Endogenous Production of Methanol after the Consumption of Fruit. *Alcoholism: Clinical and Experimental Research*, 21, 939-943.

Magnuson B. A., Burdock G. A., Doull J et al (2007). Aspartame: a safety evaluation based on current use levels, regulations, and toxicological and epidemiological studies. *Critical Reviews in Toxicology*, 37, 629-727.

Majchrowicz, E. and Mendelson, J.H. (1971). Blood Methanol Concentrations During Experimentally Induced Ethanol Intoxication in Alcoholics. *The Journal of Pharmacology and Experimental Therapeutics*, 179, 293-299.

Monte, W.C. (1984).Aspartame: Methanol and the Public Health. *Journal of Applied Nutrition*, 36, 42-54.

Morin, A.M., Liss, M. (1973). Evidence for a Methylated Protein Intermediate in Pituitary Methanol Formation *Biochem. Biophys*, *Res. Comm,* 52, 373-378 [awaiting original]

Naraqi, S., Dethlefs, R.F., Slobodniuk, R.A. *et al*, (1979). An Outbreak of Acute Methyl Alcohol Intoxication. *Aust . N.Z. Journal of Medicine*, 9, 65-68.

Röe, O. (1982). Species Differences in Methanol Poisoning. CRC Crit Rev Toxicol,10, 275-286.

Stegink, L.D., Brummel, M.C., McMartin, K.C. *et al* (1981). Blood Methanol Concentrations in Normal Adult Subjects Administered Abuse Doses of Aspartame. *The Journal of Toxicology and Environmental Health*, 7, 281-290.

Stegink, L.D., Brummel, M.C., Filer, L.J. *et al* (1983). Blood Methanol Concentrations in One Year Old Infants Administered Graded Doses of Aspartame. *The Journal of Nutrition*, 1600, 281-1606.

Stegink, L.D., Filer, L.J. Bell, E.F., *et al* (1989). Effect of Repeated Ingestion of Aspartame-Sweetened Beverage on Plasma Amino Acid, Blood Methanol, and Blood Formate Concentrations in Normal Adults. *Metabolism*, 38, 357-363.

Swartz, R.D., Millman, R.P., Billi, J.E. *et al* (1981). Epidemic Methanol Poisoning: Clinical and BiochemicalAnalysis of a Recent Episode. *Medicine*, 60, 3730382.

Taucher, J, Lagg, A, Hansel. W, *et al*, (1995). Methanol in Human Breath. *Alcoholism; Clinical and Experimental Research*, 19, 1147-1150.

Tephly, T.R. (1991). The Toxicity of Methanol. *Life Sciences*, 48, 1031-1041.

Vale, A. (2007). Methanol. *Medicine,* 35, 633-4.

Ward, K.W., Blumenthal, G.M., Welsch, F., *et al* (1997). Development of a Physiologically Based Pharmacokinetic Model to Describe the Disposition of Methanol in Pregnant Rats and Mice. Toxicol, Appl, Pharmacol, 145, 311-322.

WHO (1997). Environmental Health Criteria196: Methanol. World Health Organization, Geneva.

WHO (1999). Environmental Health Criteria 210: Principles for the Assessment of Risks to Human Health from Exposure to Chemicals. World Health Organization, Geneva.

ABBREVIATIONS

ADH	Alcohol Dehydrogenase
ADI	Acceptable Daily Intake
ATP	Adenosine Triphosphate
BW	Body Weight
CNS	Central Nervous System
DHF	Dihydrofolic acid
DINFO	Daily intake via natural Food Occurrence
EC	European Commission
EU	European Union
FSA	Food Standards Agency
mmol	Millimoles
mM	Millimolar
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NIOSH	National Institute for Occupational Health and Safety
PBPK	Physiologically Based Pharmacokinetic
PPM	Parts per million
SAM	*S*-adenosylmethionine
THF	Tetrahydrofolic Acid
VD	Volume of Distribution
WHO	World Health Organisation