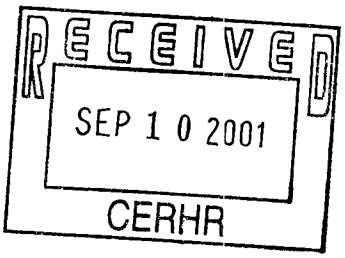
Methanol Institute

Phone: 202 / 467-5050
Fax: 202 / 331-9055



September 6, 2001

Dr. Michael Shelby
CERHR Director
NIEHS
79 T.W. Alexander Drive
Building 4401, Room 103, MD EC-32
Research Triangle Park, NC 27709

Dear Dr. Shelby:

Enclosed you will find the comments of the Methanol Institute regarding the Draft NTP-CERHR Expert Panel Report on Reproductive and Developmental Toxicity of Methanol.

Established in 1989, the Methanol Institute serves as the trade association of the methanol industry in the United States.

In addition to submitting these written comments, the Methanol Institute also wishes to provide oral testimony at the October 15th public meeting of the Expert Panel.

Thank you for this opportunity to provide comment on the Expert Panel's draft report. Please let us know if you have any questions regarding our comments.

Sincerely,

John E. Lynn
President & CEO

Enclosure

800 Connecticut Avenue, NW, Suite 620 • Washington, DC 20006 • E-mail: MI@methanol.org • Internet: www.methanol.org

COMMENTS ON

DRAFT

NTP-CERHR EXPERT PANEL REPORT ON

REPRODUCTIVE AND DEVELOPMENTAL

TOXICITY OF METHANOL

September 6, 2001

Prepared by the Methanol Institute

800 Connecticut Avenue, Suite 620 – Washington, DC 20006
(202) 467-5050 – www.methanol.org

INTRODUCTION

The draft report NTP-CERHR Expert Panel Report on Reproductive and Developmental Toxicity of Methanol is overall a well done balanced review of the data on the potential reproductive and developmental effects of methanol in several species. The comments that follow are, in general, directed to key inhalation studies. Oral studies are generally not addressed due to the high bolus doses used. They are not as relevant as inhalation studies. There are several typos that could be corrected and potential additions. These are indicated at the end of each section.

The key to the question of possible developmental or reproductive risk to humans is the interpretation of the data in different species. While the metabolism is similar in all species, some major differences in metabolic rate result in different chemical species being the ultimate toxicant in the different animal species. The potential for developmental effects appears to be the key issue. Most of the key developmental information is inhalation data in rodents and non-human primates. The significant difference in the total daily delivered doses to different species exposed to the same airborne concentration is due to the difference in minute ventilation and body weight of the species evaluated. Lethality and blindness in humans is linked to the build up of formate in the blood primarily following high oral doses of methanol. Developmental effects in rodent inhalation studies are linked to the high levels of blood methanol, not blood formate. The difference in species response is due to saturation of the different enzymes in the elimination system in these species.

1.0 CHEMISTRY, USE AND HUMAN EXPOSURE

Section 1.1.4

Page 2, 4t^{h} line, *1.500* should be *1,500*

Page 2, 13th line, *"platinum-cobalt scale, maximum 5"* should be deleted as this specification for color is listed twice

Section 1.2.1

Page 2, 1st paragraph, 3rd line, *"biomas"* should be *"biomass"*

Page 2, Table 1-2, *"Production Volume (pounds)"* should be *"Production Volume (gallons)"*

Page 4, Worst case scenario for vapor buildup in garage is related to the use of internal combustion engine vehicles not fuel cell vehicles.

Section 1.2.4

Page 7, a respiratory volume of 10 m^3 is widely used for an 8-hour workplace exposure (Snyder and Andrews 1996). 20 m^3 per day is used for 24-hour exposure. The difference is that workplace exposure is usually related to increased respiratory volume due to normal work activity.

2.0 GENERAL TOXICITY AND BIOLOGICAL EFFECTS

Section 2.1.1.1

Page 10, 4th paragraph line 12 and 13 - limit of detection of methanol in the blood is listed as 3.5 mg/l, but the value for animals treated at 34 mg/kg aspartame is reported at 3.0 +/- 1.0 mg/l (below the limit of detection???). Also see page 9, limit of detection reported as 4.0 mg/l in the same study.

Page 11, 3rd paragraph line 2 - states that a method with a lower limit of detection was used. What is the limit of detection with this method?

Page 11, 4rd paragraph line 4 – *obtained* is misspelled *(obrained)*

Page 14, *"Sedivec"* is misspelled in the heading of Table 2-1

Page 15, 4th paragraph, 8th line, the first 3 should be a 2

Page 17, 1st line there is an extra word *"authors"*

Section 2.1.1.2

Methanol blood levels in various animal species exposed by inhalation reported in Tables 7.2 C, 7-2 D, 7-2 E have been converted to mg/kg bw (total daily delivered dose) by using the following information (Kennedy 1988) and the following formula:

mg/m3 x minute ventilation x length of daily exposure divided by body weight (bw).

TABLE 1 BREATHING PATTERNS IN DIFFERENT SPECIES

Species	Body Weight (Kg))	Frequency (Breath/min)	Tidal volume (ml)	Minute Ventilation (liters)
Man	70	12-17	750-1700	9-28.9 l/min
Monkey	3	40	21	0.84 l/min
Rats	0.35	160	1.4	0.24 l/min
Mice	0.03	180	0.25	0.045 l/min
Monkey (Rhesus)[a]	*6*			*1.97 l/min*

a = Horton et al.(1992) – rhesus monkey

This conversion allows the comparison of blood methanol to total delivered daily dose in different species instead of comparing blood methanol to airborne concentration. The use of delivered dose rather than airborne concentration was also suggested by Horton et al. (1992) for high methanol exposures (above saturation of enzyme systems). It also corrects for differences in the length of exposure (2.5 hours for Burbacher et al. [1999 b] to 21 hours plus for the NEDO 1996/1997data). By calculating the total delivered daily dose, comparison between species and within species can be made. The calculations are simple. They assume minute ventilation rates are the same at high and low exposure and no attempt was made to correct for clearance, which could be quite different (2.5 vs. 23 hrs exposure per day). The correlation between blood methanol and total delivered daily dose (mg/kg bw) is much better than the correlation between airborne concentration and blood methanol. Metabolic conversion of methanol in different species is saturable at high doses, with a half-life ranging up to 1 day for doses in excess of 1 g/kg (Kavet and Nauss 1990). The blood methanol data in the rodent demonstrates that metabolic conversion is saturated. Pharmacokinetic models predict that differences in blood methanol concentrations are not large in rodents and humans exposed to methanol at low exposure concentrations (Ward et al. 1997, Horton et al. 1992). A similarity in actual response in various species can be seen following low inhalation doses (Table 2). At 1000 ppm the predicted blood methanol concentration in mice is 3-7 fold greater than that in humans, while exposures to 5000 ppm will result in blood methanol levels 13-18 fold greater in mice than humans (Perkins et al. 1995a).

TABLE 2 TOTAL DAILY DELIVERED DOSE AND BLOOD METHANOL LEVELS IN DIFFERENT SPECIES FOLLOWING INHALATION EXPOSURE[a]

Dose Mg/kg	Exposure	Species	Blood Methanol (mg/l)	Comments	Reference
11	200 ppm – 6 hrs	Rat	3.1		Horton et al. 1992
11	200 ppm –2.5 hrs	Monkey	4.7-5.5	NOAEL	Burbacher et al. 1999a
19	200 ppm – 4 hrs	Human	6.5	Formate 11.2 mg/l	Osterloh et al. 1996
27	200 ppm – 6 hrs	Human	7-8	Formate 9.5 mg/l	Lee et al. 1992
31	200 ppm – 6 hrs	Monkey	3.9		Horton et al. 1992
33	600 ppm – 2.5 hrs	Monkey	10.5-11	NOAEL	Burbacher et al.1999a
98	1800 ppm –2.5 hrs	Monkey	36	NOAEL, Formate ~15 mg/l	Burbacher et al. 1999a
133	800 ppm – 8 hrs	Humans	31		Batterman et al.1998
184	1200 ppm - 6 hrs	Monkey	38	Formate –13.2 mg/l ?	Horton et al. 1992
308	2000 ppm – 6 hr	Monkey	64	Formate –13.2 mg/l ?	Horton et al. 1992
385	1200 ppm – 6 hrs	Rat	27		Horton et al. 1992
428	1000 ppm – 8 hrs	Rat	83		Pollack & Brouwer 1996
642	2000 ppm – 6 hrs	Rat	80	Formate –13.2 mg/l ?	Horton et al. 1992
819	1000 ppm – 7 hrs	Mouse	97	NOAEL	Rogers et al. 1993
1375	3000 ppm – 21 hrs	Monkey	80	Formate 30 mg/l	NEDO 1986/1987
1444	4500 ppm – 6 hrs	Rat	550		Stern et al.1996
1638	2000 ppm – 7 hrs	Mouse	537	Fetal LOAEL	Rogers et al. 1993
1869	5000 ppm – 7 hrs	Rat	1000-2170	Fetal NOAEL	Nelson et al. 1985
2139	5000 ppm – 8 hrs	Rat	1047		Pollack & Brouwer 1996
2293	5000 ppm – 21 hrs	Monkey	5250	Formate 1210 mg/l	NEDO 1986/1987
2340	25000 ppm – 8 hrs	Mouse	1883		Pollack & Brouwer 1996
3738	10000 ppm – 7 hrs	Rat	1840-2240	Maternal NOAEL Fetal LOAEL	Nelson et al. 1985
4095	5000 ppm – 7 hrs	Mouse	1650		Rogers et al. 1993
4280	10000 ppm – 8 hrs	Rat	1656		Pollack & Brouwer 1996
4680	5000 ppm – 8 hrs	Mouse	3580		Pollack & Brouwer 1996
5616	15000 ppm – 7 hrs	Rats	3169-3826		Stanton et al. 1995
6143	7500 ppm – 7 hrs	Mouse	3178		Rogers et al. 1993
6420	15000 ppm – 8 hrs	Rat	2667		Pollack & Brouwer 1996
7020	10000 ppm – 6 hrs	Mouse	2080		Dorman et al. 1995
7476	20000 ppm – 7.5 hrs	Rat	5250-8650		Nelson et al. 1985
8190	10000 ppm – 7hrs	Mouse	4204		Rogers et al. 1993
8560	20000 ppm – 8 hrs	Rat	3916		Pollack & Brouwer 1996
9360	10000 ppm – 8 hrs	Mouse	6028		Pollack & Brouwer 1996
10530	15000 ppm – 6 hrs	Mouse	7130		Dorman et al.1995
12285	15000 ppm –7 hrs	Mouse	7330		Rogers et al. 1993
14040	150000 ppm – 8 hrs	Mouse	11165		Pollack & Brouwer 1996

a= Inhalation data from table 7.2 C, 7-2 D, 7-2 E in the draft report and data from NEDO (1986/1987) has also been added

In the monkey formate metabolism is reported to saturate at about 250 mg/kg [bolus dose?] according to a formula derived by the HEI and cited in Horton et al. (1992). Horton et al.. (1992) exposed rhesus monkeys to methanol by inhalation between 50 and

2000 ppm (8 – 308 mg/kg) for 6 hours. The formate levels were not statistically different in the control and exposed monkeys (2000 ppm, 308 mg/kg). Reynolds et al. (1984) and Suomi (1984) evaluated infant monkeys given various doses of aspartame up to 2500-2700 mg/kg (250-270 mg/kg methanol) for effects on growth, some hematological and blood measurements including pH, and behavioral effects. The NOAEL was 250-270 mg/kg methanol, the highest dose tested. These results suggest formate metabolism is not saturated at 250-270 mg/kg as suggested by the HEI formula. Saturation of formate metabolism appears to be much higher in inhalation studies in monkeys. This conclusion is based on blood formate levels. There appears to be saturation of the enzyme system between 1375-2293 mg/kg bw based on data from monkeys exposed at 3000 – 5000 ppm- 21 hours per day (NEDO 1986/1987).

Blood methanol levels determined by Horton et al. (1992) were linearly related to airborne concentrations below 1200 ppm (184 mg/kg) but not at 2000 ppm (308 mg/kg), suggesting the beginning of saturation of the alcohol dehydrogenase enzyme system in Rhesus monkeys. Saturation of methanol metabolism, based on blood methanol levels (5250 mg/l at 5000-ppm vs 80 mg/l at 3000 ppm), appears to be between 1375-2293 mg/kg based on data from cynomolgus monkeys exposed for 21 hours per day (NEDO 1986/1987).

There are several inhalation studies in mice exposed to different airborne concentrations for varying lengths of daily exposure. A comparison of the total daily delivered dose to the blood methanol levels in these mouse inhalation studies is seen in Table 3.

TABLE 3

COMPARISON IN MICE OF TOTAL DAILY DELIVERED DOSE TO BLOOD METHANOL LEVELS

Dose mg/kg	Exposure Conditions	Blood methanol (mg/l)	Comments	Reference
819	1000 ppm – 7 hrs	97	NOAEL	Rogers et al. 1993
1638	2000 ppm – 7 hrs	537	Fetal LOAEL	Rogers et al. 1993
2340	2500 ppm – 8 hrs	1883		Perkins et al. 1995b
4095	5000 ppm – 7 hrs	1650		Rogers et al. 1995b
4680	5000 ppm – 8 hrs	3580		Perkins et al. 1995b
6143	7500 ppm – 7 hrs	3178		Rogers et al. 1993
7020	10000 ppm – 6 hrs	2080		Dorman et al. 1995
8190	10000 ppm – 7hrs	4204		Rogers et al. 1993
9360	10000 ppm – 8 hrs	6028		Perkins et al. 1995b
10530	15000 ppm – 6 hrs	7136		Dorman et al. 1995
12285	15000 ppm –7 hrs	7330		Rogers et al. 1993
14040	15000 ppm – 8 hrs	11165		Perkins et al. 1995b

There are also several inhalation studies in monkey exposed to different airborne concentrations for various lengths of daily exposure. A comparison of the total daily delivered dose to the blood methanol and formate level in the monkey inhalation studies is seen in Table 4.

TABLE 4

COMPARISON IN MONKEYS OF TOTAL DAILY DELIVERED DOSE TO BLOOD METHANOL LEVELS

Dose mg/kg	Exposure Conditions	Blood methanol (mg/l)	Comments	Reference
11	200 ppm –2.5 hrs	5	NOAEL	Burbacher et al. 1999a
31	200 ppm – 6 hrs	3.9	Formate –13.2 mg/l ?	Horton et al. 1992
33	600 ppm – 2.5 hrs	11	NOAEL	Burbacher et al. 1999a
98	1800 ppm –2.5 hrs	35	NOAEL, Formate ~15 mg/l	Burbacher et al. 1999a
184	1200 ppm - 6 hrs	38	Formate –13.2 mg/l ?	Horton et al. 1992
308	2000 ppm – 6 hrs	64	Formate - 13.2 mg/l?	Horton et al. 1992
1375	3000 ppm –21 hrs	80	Formate - 30 mg/l	NEDO 1986/1987
2293	5000 ppm – 21 hrs	5250	Formate - 1210 mg/l	NEDO 1986/1987

Suggested Corrections and Additions

The NEDO (1986,1987) study should be included in the draft report as it contains useful information. Together the reports contain 412 pages of information on methanol. In the NEDO 1987 report (p 51) methanol and formate levels were reported in monkeys exposed at 3000 ppm (21 days for 21 hours per day) and 5000 ppm (14 days for 21 hours per day). The monkeys exposed at 5000 ppm (2293 mg/kg) showed greatly elevated methanol and formate levels (methanol 5250 mg/l, formate 1210 mg/l) while little effect was noted at 3000 ppm (1375 mg/kg) (methanol 80 mg/l, formate 30 mg/l). This suggests saturation of the enzymes involved in converting formate to carbon dioxide between 3000 ppm (1361 mg/kg) and 5000 ppm (2268 mg/kg) in the monkey. NEDO 1987 also reported on an intraperitoneal injection study of methanol (25, 125, 600 and 3000 mg/kg). They measured blood methanol, blood formate, blood pH, urinary, fecal and expiration excretion over 8 times 48 hours after injection. Effects were seen at 3000 mg/kg. This suggests saturation of formic dehydrogenase between 600 mg/kg and 3000 mg/kg in the cynomolgus monkey. NEDO concluded that rats and monkeys behaved similarly at levels up to 600 mg/kg bw.

2.2.2

Page 37, exposure in the NEDO study was *20 hours a day* not *2 hours* as stated in the draft report.

Page 38, reference to Andrews and Terrill (1987) should be Andrews et al. (1987)?

2.3

Page 43, heading of table 2.9 - remove the period at end of the title

2.4

There are 3 published studies that assess to some degree carcinogenicity in animals (2 skin painting and a drinking water study). Methanol was used in one or more of the control groups in these studies (See Forbes, P.D.. Urbach, F and R.E. Davies (1979)

Enhancement of experimental photocarcinogenesis by topical retinoic acid. *Cancer Letters.* 7 85-90 85 and Apaja, M, (1980) Evaluation of toxicity and carcinogenicity of malonaldehyde *Acta Univ. Oul D55. Anat. Pathol Microbiol.*).

3.0 DEVELOPMENTAL TOXICITY DATA

3.1 Human data

Page 49, second to last line replace *"of"* with *"or."*

The question of folate deficiency in humans as it relates to methanol is complex. Folate deficiencies in humans have resulted in adverse developmental effects. Folate is involved in the conversion of formate. Based on adverse effects in humans following methanol exposure formate appears to be the primary toxic metabolite in humans. This suggest that folate deficiency and methanol exposure are not a good combination, but there is no adequate data in humans to answer if this combination of folate deficiency and methanol causes developmental effects in humans (Lorente et al. 2000).

In rodent studies where developmental effects are noted in high dose studies, the toxicity appears to be much more closely related to high methanol blood levels than to any increase in blood formate levels. The few animal studies with folate deficient rodents or that added formate in embryo culture at a very high dose show some effect, but it does not appear that formate plays a major causal role in rodents developmental studies with methanol (Sakanashi et al. 1996, Fu et al. 1996. Dorman et al. 1995). Methanol itself appears to be the primary toxic agent in rodent developmental studies.

3.2.1 Prenatal Development

Rats

In the Nelson et al. (1985) study rats were exposed to very high concentrations of methanol by inhalation (5000, 10000, 15000 ppm). Based on the data from Horton et al. (1992) the start of enzymatic saturation in rats was noted between 1200 –2000 ppm [385-642 mg/kg]. Horton et al. (1992) data supports the saturation on the catalase enzyme at all levels tested by Nelson et al. (1992). The results of this high dose study show a LOAEL for fetal effects at 10000 ppm with a NOAEL of 5000 ppm. Converting ppm to total daily delivered dose in mg/kg bw per day for the rat in the Nelson et al. study indicates that all tested doses, including the LOAEL, are greater than the lethal dose in humans (see table 5). The relevance of this study to developmental effects in humans is limited. The metabolism is saturated, and the enzyme that is involved in the first step of elimination is different than the enzyme involved in the first step with humans. The ultimate toxin appears to be related to high blood methanol levels not the blood formate levels. The levels tested are much higher than would be likely seen in a human exposure situation.

TABLE 5

RESPONSE IN RATS COMPARING TOTAL DAILY DELIVERED DOSE AND BLOOD METHANOL TO LETHAL DOSE IN HUMANS (NELSON ET AL.)

Total dose (mg/kg)	Exposure conditions	Blood methanol (mg/l)	Comment	Ratio of total dose in rats to lethal dose humans (300-1000 mg/kg)
1869	5000 ppm (6500 mg/m3) for 7 hours	1000-2170	NOAEL	~2 – 6
3738	10000 ppm (13000 mg/m3) for 7 hours	1840-2210	LOAEL – Develop	~4- 12
7476	20000 ppm (26000 mg/m3) for 7 hours	5250 -8650	LOAEL – Maternal	~7 – 25

Suggested Corrections and Additions

An unpublished rat study conducted by NEDO (1986) should be mentioned in more detail in the report. Rats were exposed during gestational days 7-17 to 200, 1000, or 5000 ppm for 22.7 hours/day (equal to 241, 1214, or 6061 mg/kg bw*). [Based on a daily exposure of 7 hours per day as in the Nelson et al. 1985 study the levels used in the NEDO study would be equivalent to 600, 3000 and 15000 ppm]* A no-observable-adverse-effect level (NOAEL) of 1000 ppm (1214 mg/kg) methanol was determined in the NEDO study. Pregnant rats in the NEDO study exposed at 5000 ppm (6061 mg/kg) showed a reduced body weight gain, as well as, reduced food and drinking water consumption. An increase in embryo death rate and teratogenic effects were observed in the 5000 ppm (6061 mg/kg) group. An increase in the length of gestation (0.7 day) was observed in the 5000 ppm (6061 mg/kg) group. Nelson et al. (1985) observed a NOAEL of 5000 ppm (1863 mg/kg) and a LOAEL of 10000 ppm (3738 mg/kg) in rats exposed for a longer period during gestation (days 1-19), but shorter daily exposure duration (7 hours/day). The two studies together suggest a NOAEL of 1863 mg/kg or greater and a LOAEL of 3738 mg/kg or lower in rats.

Mice

In the Rogers et al. (1993) study mice were exposed to methanol by inhalation. The data supports the saturation of the catalase enzyme at all levels above 1000 ppm. The results of this dose study show a LOAEL for fetal effects at 2000 ppm with a NOAEL of 1000 ppm and support the idea that the mouse is the most sensitive species. As with the rat, converting ppm to total daily delivered dose in mg/kg per day for the effect levels in the mouse gives mg/kg bw doses that are greater than the lethal dose in humans (Table 6). The relevance of this study to developmental effects to humans is limited. The metabolism is saturated at the effect levels and the enzyme that is involved in the first step of elimination is different than the enzyme involved in the first step with humans. The ultimate toxin appears to be related to high blood methanol levels not the blood formate levels. The effect levels tested are much higher than what would likely have

been seen in a human exposure situation based on the total daily delivered mg/kg bw dose.

TABLE 6

RESPONSE IN MICE COMPARING TOTAL DAILY DELIVERED DOSE AND BLOOD METHANOL TO LETHAL DOSE IN HUMANS (ROGERS ET AL.)

Total dose (mg/kg)	Exposure conditions	Blood methanol (mg/l)	Effect	Ratio of total dose in mice to lethal dose humans (300-1000 mg/kg)
819	1000 ppm (1300 mg/m3) for 7 hours	97	NOAEL	~1
1638	2000 ppm (2600 mg/m3) for 7 hours	537	LOAEL Develop- ribs	~ 1.6 – 5.5
4095	5000 ppm (6500 mg/m3) for 7 hours	1650		4 – 14
6142	75000 ppm (9750 mg/m3) for 7 hours	3178		6 – 20
8190	10000 ppm (13000 mg/m3) for 7 hours	4204		8 – 27
12285	15000 ppm (19500 mg/m3) for 7 hours	7330		12 – 41

If the catalase enzyme system, which is primarily responsible in rats and mice for converting methanol to formaldehyde, is saturated then there may be an increase in oxygen species because of reduced catalase activity. It has been reported that an inhibition of catalase in the mouse embryo produces a significant increase in malformations supporting the idea that oxidative stress may play a key role in certain kinds of birth defects (Bauman et al. 1996, Winn and Wells 1999). The addition of catalase to embryo cultures block embryotoxicity suggesting that reactive oxygen species play a role in some teratogenic responses (Wells et al. 1997). The potential role of reactive oxygen species in rodents exposed to methanol is a question that requires further evaluation. If reactive oxygen species are a major factor in the response in rodents, and this response is related to the catalase pathway, not the alcohol dehydrogenase pathway, then the rodent studies are not useful in predicting human developmental effects.

Suggested Corrections and Additions

Page 55, 4th paragraph the statement *" methanol concentrations were dose related, did not appear to reach saturation---"* needs clarification. Absorption was not saturated, but metabolism appears to be saturated. Blood methanol concentration increases 5 fold from 1000 ppm to 2000 ppm (see Table 6) supporting saturation of the catalase enzyme between 1000 ppm (819 mg/kg bw) and 2000 ppm (1638 mg/kg bw).

3.2.2 Postnatal

Monkey

The Burbacher et al. (1999 a, b) study in monkeys is well done. The top dose used was calculated to be close to 100 mg/kg bw (Table 6). This dose is well below a bolus dose (250 mg/kg bw) that would result in the build up of formate. The blood formate measurements support this lack of accumulation. Methanol levels in the blood shoe a small increase, but the increase does not appear to cause any adverse effects. Formate, the primary toxic metabolite in humans and monkeys, is the major concern in methanol poisoning in humans. The Burbacher et al. (1999 b) study reports a decrease in the gestation time, but no dose response was noted. The shortening of gestation length appears to have been a result of C-sections preformed in several treated monkeys. Gestation time in this study was still in the normal range and no effect on birth weights was noted suggesting that this was not a treatment-related effect. It should be noted that a decrease in length of gestation has not been observed in any other developmental studies, but NEDO (1986/1987) reports in a rat teratology study that the length of gestation was increased at the top dose of 5000 ppm (daily exposure time is 3 times longer than was used in the Nelson et al (1985) study ie 5000 ppm in the NEDO study equal to 15000 ppm in the Nelson et al. 1985 study).

Another observation was the wasting syndrome reported in 2 offspring from dams that were exposed at 1800 ppm. In a study where young primates received aspartame for 9 months (up to 270 mg/kg bw of methanol per day), no treatment related effects were reported. The lack of treatment related effects at a higher daily dose than used in the Burbacher et al. (1999 b) study supports the suggestion that the wasting syndrome noted is a chance observation and not related to treatment.

Behavioral testing produced two effects that might suggest an effect of methanol exposure. The responses were small, as was the number of animals. This suggests that a random occurrence may be as likely a cause as is methanol exposure. In comments prepare for AP&FA by several experts concerning this issue it was concluded that these behavioral effects are "highly unlikely" and unlikely to be real." Detailed comments prepared by various experts concerning the Burbacher et al. (1999 b) study address the decrease in gestation time, the wasting effect and some behavioral observations. These comments submitted to EPA on July 3, 2000 by AF&PA appear to be very convincing, and agree with the study's authors that no clear-cut treatment related effects were demonstrated in the Burbacher et al, (1999 b) study.

In a study where young primates received aspartame for 9 months (up to 270 mg/kg bw of methanol per day), no treatment related behavioral effects were reported (Reynolds et al. 1984). Suomi (1984) tested these monkeys at 1.5 years of age and no effects on learning tests or hearing ability were noted. The lack of treatment related effects at a higher daily dose than used in the Burbacher et al. (1999 b) study supports the suggestion that the behavioral effects noted in the Burbacher et al. (1999 b) study are random observations and not related to treatment.

In a limited study conducted by Stanton et al. (1995) rats exposed to 15000 ppm (5616 mg/kg/bw) were evaluated for behavioral effects. No treatment related behavioral effects were noted in the offspring. The Weiss et al. (1996) inhalation study exposed rats to 4500 ppm and also evaluated behavioral effects. This study got isolated positive

responses that were small and inconsistent. The significance of these responses has to be considered questionable.

Suggested Corrects and Additions

Page 64, 1st paragraph, 8th line, *"does necessarily"* should be *"does not necessarily."*

Page 65, 3rd paragraph, 10th line, *"separation this"* should be *"separation in this."*

Page 66, 3rd paragraph, 5th line, *"studied"* should be *"study."*

Page 67, *2.5-2.7 mg/kg/day* should be *2.5-2.7 g/kg /day.*

Page 76, 3rd paragraph, 19th line, *"increased"* should be *"increase."*

Page 83, 1st paragraph, 9th line, *"concluded"* should be *"conclude"*; 5th paragraph, 5th line, *"reduced"* should be *"reduce"*; and 5th paragraph, 16th line, *"then"* should be *"than."*

Table 3-4 p 8 7 draft report ([redacted]

SPECIES	MATERNAL NOAEL	FETAL NOAEL	MATERNAL LOAEL	FETAL LOAEL
RAT	10,000 ppm [redacted] 1840-2240 mg/l methanol	5,000 ppm [redacted] 1000-2170 mg/l methanol	20,000 ppm [redacted] 5250-8650 mg/l methanol	10,000 ppm [redacted] 1840-2240 mg/l methanol
MOUSE	15,000 PPM [redacted] 7,330 mg /l methanol	1000 PPM [redacted] 97 mg/kg bw	UNKNOWN	2,000 PPM [redacted] 537 mg/l methanol

4.0 REPRODUCTIVE TOXICITY

Suggested Corrections and Additions

The NEDO (1987) study should be included in the draft report as useful information about reproductive performance. NEDO conducted a 2-Generation inhalation toxicity study in rats. Rats were exposed at 10, 100 or 1000 ppm 19-21 hrs per day (11, 106,

1070 mg/kg bw). No effects were reported in the 10, 100 ppm groups. Males and to a lesser degree females, in the 1000ppm (1070 mg/kg) showed a reduced body weight gain. Post-natal morphological examination of males showed a slightly earlier descensus testis in the 1000 ppm group (1070 mg/kg bw). No change attributable to methanol was observed in movement function test as a reflex reaction, emotional or learning test. Animals in the high-exposure group (1000 ppm – 1070 mg/kg) showed a significant decrease in brain weight, but no histopathological effects. No effects on reproduction, pregnancy and delivery period were noted.

4.0 CRITICAL DATA NEEDS

Species differences

The toxic metabolite in man and monkeys is believed to be formate. Limited levels of formaldehyde dehydrogenase in these species result in a build up of formate if the methanol exposure is high enough. Developmental effects have only been demonstrated in rodents exposed to high levels of methanol. The total daily delivered dose of methanol that produces effects in rodents is greater than the minimal lethal dose in humans. In the case of the rodents the agent causing developmental effects appears to be a high blood level of methanol. The mouse is more sensitive than the rat with a ~3 fold higher blood methanol level at the same airborne concentration. The primary enzyme in the conversion of methanol to formaldehyde in the body is different in the rodent (catalase) and humans/monkey (alcohol dehydrogenase). The rodent effects are noted only after the catalase pathway is saturated. This leads to questions about the specific role that the saturation of the catalase system may have (increase in reactive oxygen). The species difference in metabolism, the difference in the actual toxic agent, and the high doses necessary to produce an effect raises questions about the value of these rodent studies in predicting developmental concerns in humans. Addition research could help explain the mechanism of action in the rodent, but it would not necessarily be relevant to humans.

The question of folate deficiency in humans as it relates to methanol is complex. Formate appears to be the primary toxic metabolite in humans exposed to methanol. This suggest that folate deficiency and methanol exposure are not a good combination, but there is no adequate data in humans to answer if this combination of folate deficiency and methanol causes developmental effects in humans. In rodent studies where developmental effects are noted, toxicity appears to be much more closely related to high methanol blood levels than to an increase in blood formate levels. Is elevated blood formate a potential concern from a developmental point of view? Most likely not, the effect on blood pH, blindness and death are the primary concerns in human with elevated blood formate.

The relevance of these developmental studies in rodents to humans is limited. In the rodent the metabolism is saturated: the enzyme in the first step is different from that used by humans, and the ultimate toxin appears to be related to high blood methanol levels and not the blood formate levels. The levels tested in rodent studies are much higher than those likely to be seen in a human exposure situation. The total daily delivered dose in mg/kg bw in these rodent studies would be lethal to humans.

Overall Conclusion

Methanol causes developmental effects in rodents exposed at high doses, but no developmental effects have been demonstrated in humans or nonhuman primates. The relevance of these developmental studies in rodents to humans is limited. There is limited data available on potential reproductive effects, but there is no indication that methanol causes a reproductive hazard. Methanol is not considered a developmental or reproductive hazard to humans.

REFERENCES

American Forest & Paper Association, Inc. (AF&PA) comments to EPA July 3, 2000 petition to remove methanol from list of hazardous air pollutants

Battennan SA, Franzblau A, DArcy JB, Sargent NE, Gross KB, Schreck RM. Breath, urine, and blood measurements as biological exposure indices of short-term inhalation exposure to methanol. Int Arch Occup Environ Health 71:325-35(1998).

Bauman JW, Denno KM, Taylor BB, and Sadler TW. Developmental toxicity induced by antisense inhibition of catalase in cultured mouse embryo, Teratology 53:84 (1996)

Burbacher T, Shen D, Grant K, Sheppard L, Daniian D, Ellis S, Liberatc) N. HEI Research Report Number 89: Part 1: Methanol disposition and reproductive toxicity in adult females. In: Reproductive and offspring developmental effects following maternal inhalation exposure to methanol in nonhuman primates., vol 1. Seattle, Washington-Health Effects Institute -- HEI, 1999 a.

Burbacher T, Grant K, Shen D, Damian D, Ellis S, Liberato N. HEI Research Report Number 89: Part 11: Developmental effects in infants exposed prenatally to methanol. In: Reproductive and offspring developmental effects following maternal inhalation exposure to methanol in nonhuman primates., vol 11. Seattle, Washington:Health Effects Institute -- HEI, 1999 b.

Dorman DC, Bolon B, Struve MF, LaPerle KM, Wong BA, Elswick B, Welsch F. Role of formate in methanol-induced exencephaly in CD-1 mice. Teratology 52:30-40(1995).

Dorman DC, Moss OR, Farris GM, Janszen D, Bond JA, Medinsky MA, Pharmcokinetics of Inhaled (I 4C) Methanol and Methanol-Derived 14-C Formate in normal and-deficient cynomolgus monkeys. Toxicol. Appl Pharmacol 128:229-238(1994).

Fu SS, Sakanashi TM, Rogers JM, Hong KH, Keen CL. Influence of dietary folic acid on the developmental toxicity of methanol and the frequency of chromosomal breakage in the CD-1 mouse. Reprod Toxicol 10:455- 63(1996).

Horton VL, Higuchi MA, Rickert DE. Physiologically based pharmacokinetic model for methanol in rats, monkeys, and hurnans. Toxicol Appl Pharmacol 117:26-36(1992).

Kavet R, and Nauss K. The toxicity of inhaled methanol vapors. Crit Rev Toxicol 21:21-50(1990).

Kennedy GL , Inhalation toxicology in Principles and Methods of Toxicology- Hayes W, ed., 2 nd edition p, Raven Press New York (1988) 361.

Lorente C, Cordier S, Bergeret A, De Walle RE, Goujard J, Ayme S, Knill-Jones R, Calzolari E, Bianchi F. Maternal occupational risk factors for oral clefts. Scand J Work Environ Helth 26:137145(2000).

Lee EW, Terzo TS, DArcy JB, Gross KB, Schreck RM. 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(1992).

NEDO Toxicological research on methanol as a fuel for power stations, (Sept. 1986)

NEDO Toxicological research on methanol as a fuel for power stations, A summary report on test with monkeys, rats and mice, (Oct. 1987).

Nelson BK, and Brightwell WS. Teratological assessment of methanol and ethanol at high inhalation levels in rats. Fund Appl Toxicol 5:727-36(1985).

Osterloh JD, D'Alessandro A, Chuwers P, Mogadeddi H, Kelly TJ. Serum concentrations of methanol after inhalation at 200 ppm. J Occup Environ Med 38:571-6(1996).

Perkins RA, Ward KW, Pollack GM. A pharmacokinetic model of inhaled methanol in humans and comparison to methanol disposition in mice and rats. Environ Health Perspect 103:72633(1995 a).

Perkins RA, Ward KW, Pollack GM. Comparative toxicokinetics of inhaled methanol in the female CD-1 mouse and Sprague-Dawley rat. Fundam Appl Toxicol 28:245-54(1995 b).

Pollack GM, Brouwer KL. HEI Research report: Maternal-fetal pharmcokinetics of methanol 74. Chapel Hill: Health Effects institute, 1996.

Reynolds WA, Bauman AF, Naidu S, Stegnink LD, Filer LJJ. Developmental assessment of infant macques receiving dietary aspartame or phenylalanine. In: Aspartame: Physiology and Biochemistry. Marcel Dekker (Stegink LD,Filer, L J Jr, ed). New York, NY, 1984;405-424.

Rogers JM, Mole ML, Chemoff N, Barbee BD, Turner Cl, Logsdon TR, Kaviock RJ. The developmental toxicity of inhaled methanol in the CD-1 mouse, with quantitative dose-response modeling for estimation of benchmark doses. Teratology 47:175-88(1993).

Sakanashi TM, Rogers JM, Fu SS, Connelly LE, Keen CL. Influence of folate status on the developmental toxicity of methanol in the CD-1 mouse. Teratology 54:198-

206(1996).

Snyder R, and Andrews LS, Toxic effects of solvents and vapors in Cassert & Doull's Toxicology, the basic science of poisons- 5th edition, McGraw- Hill, New York (1996). 756

Stanton ME, Crofton KM, Gray LE, Gordon CJ, Boyes WK, Mole ML, Peele DB, Bushnell PJ. Assessment of offspring development and behavior following gestational exposure to inhaled methanol in the rat. Fundam Appl Toxicol 28:100-110(1995).

Stern S, Reuhl K, Soderholm S, Cox C, Sharma A, Balys M, Gelein R, Yin C, Weiss B, Perinatal methanol exposure in rats. I. Blood methanol concentration and neural cell adhesion molecules. Fundam Appl Toxicol 34:36-46 (1996).

Suomi SJ. Effects of aspartame on the learning test performance of young stump-tailed macaques. In: Aspartame: Physiology and Biochemistry (Stegink, L D; Filer, L J Jr, eds). Marcel Dekker New York, NY, 1984;425-445.

Ward KW, Blumenthal GM, Welsch F, Pollack GM. Development of a physiologically based pharmacokinetic model to describe the disposition of methanol in pregnant rats and mice. Toxicol Appl Pharmacol 145:311-22(1997).

Weiss B, Stem S, Soderholm SC, Cox , C Sharma A, Inglis GB, Preston R, Balys K Reuhl KR, Gelein R. HEI Report: Developmental Neurotoxicity of Methanol Exposure by Inhalation in rats Research Report Number 73. Cambridge, MA: Health Effects Institute, 1996.

Wells PG, Kim PM, Loposa RR, Nicol CJ, Parman T, Winn LM, Oxidative damage in chemical teratogenesis. Mutat Res 396(1-2):65-78 (1997)

Winn LM, and Wells PG, Maternal administration of superoxide dismutase and catalase in phenytoin teratogenicity. Free Radic Biol Med 26(3-4):266-74 (1999)