CHEMICAL UNDER CONSIDERATION FOR POSSIBLE LISTING VIA THE AUTHORITATIVE BODIES MECHANISM: METHANOL

June 2007

Reproductive and Cancer Hazard Assessment Section
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Methanol may meet the criteria for listing as known to the State to cause reproductive toxicity under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Health and Safety Code Section 25249.5 et seq.), more commonly known as Proposition 65, via the authoritative bodies mechanism. The regulatory requirements for listing by this mechanism are set forth in Title 22, California Code of Regulations section12306[1]. The regulations include the criteria for evaluating the documentation and scientific findings by the authoritative body that the Office of Environmental Health Hazard Assessment (OEHHA) uses to determine whether listing under Proposition 65 is required.

The National Toxicology Program (NTP), solely as to final reports of the National Toxicology Program's Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR), is one of five institutions that have been identified as authoritative bodies for identification of chemicals as causing reproductive toxicity for the purposes of Proposition 65 (Section 12306(1)(3)). NTP has identified methanol as causing reproductive toxicity. OEHHA has found that this chemical appears to be "formally identified" by NTP as causing reproductive toxicity as required by Section 12306(d) because methanol is the subject of a report published by NTP that concludes that the chemical causes reproductive toxicity and specifically and accurately identifies the chemical, and the document meets one or more of the criteria required by Section 12306(d)(2).

OEHHA also finds that the scientific criteria in Section 12306(g)(2) for "as causing reproductive toxicity" appear to have been satisfied for methanol, in that "studies in experimental animals indicate that there are sufficient data, taking into account the adequacy of the experimental design and other parameters such as, but not limited to, route of administration, frequency and duration of exposure, numbers of test animals, choice of species, choice of dosage levels, and consideration of maternal toxicity, indicating that an association between adverse reproductive effects in humans and the toxic agent in question is biologically plausible." In making this evaluation, OEHHA relied upon the discussion of data by NTP when it made its finding that methanol causes reproductive toxicity. A brief discussion of the relevant reproductive and developmental

[1] All further references are to Title 22 of the California Code of Regulations unless otherwise indicated.

toxicity studies providing evidence for the NTP findings is presented below. Much of the discussion is taken verbatim from the NTP-CERHR (2003) report *NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Methanol*. The statement quoted below in bold reflects data and conclusions that appear to satisfy the criteria for the sufficiency of evidence for reproductive toxicity in Section 12306(g)). The full citation for the NTP document is given later in this document.

Chemical Under Consideration for Possible Listing as Known to the State to Cause Reproductive Toxicity

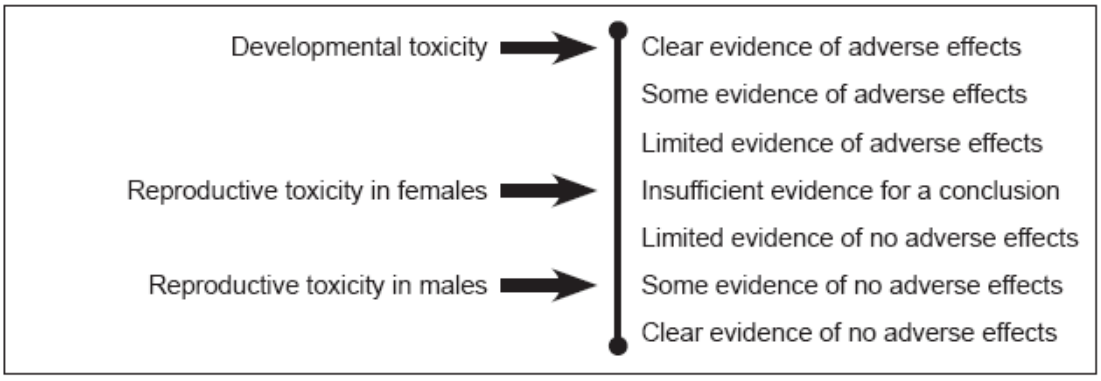
Chemical	CAS No.	Toxicological Endpoints	Chemical Use	Reference
Methanol	67-56-1	developmental toxicity	Contained in products such as varnishes, shellacs, paints, windshield washer fluid, antifreeze, adhesives, deicers, and Sterno™ heaters. Methanol vapor may also be present in cigarette smoke at a level of 180 µg/cigarette.	NTP-CERHR (2003)

Methanol (CAS No. 67-56-1).

" [E]xposure to 2,000 ppm [methanol] resulted in a significant increase in cervical ribs in the fetuses. Higher exposures significantly increased the incidence of cleft palates, exencephaly, and skeletal malformations" (NTP-CERHR, 2003, pp. 2)).

The NTP-CERHR has concluded that there is clear evidence of adverse effects for reproductive toxicity (developmental endpoint) in laboratory animals (NTP-CERHR, 2003, Figure 2).

Figure 2. *The weight of evidence that methanol causes adverse developmental or reproductive effects in laboratory animals*



The NTP-CERHR monograph says "Laboratory animal studies reviewed by the expert panel, and an additional published study using cultured mouse embryos, show that methanol can adversely affect development. …. In this case, recognizing the lack of human data and the clear evidence of laboratory animal effects, the NTP judges the scientific evidence sufficient to conclude that methanol may adversely affect human development if exposures are sufficiently high." (NTP-CERHR, 2003, p. 2).

The *NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Methanol* by the NTP-CERHR Methanol Expert Panel is incorporated into NTP-CERHR (2003) as Appendix II. The Expert Panel reviewed studies of methanol in that report, and some of the information reported for those studies, mostly taken verbatim from NTP-CERHR (2003, Appendix II), is summarized below. In addition, an *in vitro* study not available to the Expert Panel was summarized in the *NTP Monograph*, and excerpts from that summary are also given below.

As part of an effort to assess teratogenic effects of industrial alcohols, Nelson et al. (1985) studied the effects of prenatal methanol exposure in Crl: Sprague-Dawley rats. Nelson et al. exposed 15 pregnant rats per group to 0, 5,000, 10,000, or 20,000 ppm methanol (99.1 percent purity; nominal concentrations) in air for 7 hours/day (Table 7.3-A). The two lower dose groups were exposed on gd 1–19 whereas the 20,000 parts per million (ppm) group was exposed on gd 7–15. Two groups of 15 control rats (one for the 10,000 and 20,000 ppm group and one for the 5,000 ppm groups) were exposed to air only. Blood methanol levels in concurrently-exposed, non-pregnant rats on days 1, 10, and 19 of exposure were measured by GC at 1,000 –2,170, 1,840 –2,240, and 5,250 – 8,650 mg/L in the low- to high-dose group, respectively. Background levels of blood methanol were not provided. The study authors assumed that blood methanol levels in pregnant rats were similar to those determined in non-pregnant rats. Maternal toxicity was evidenced by a slightly unsteady gait only in the high dose group during the first few days of exposure; there were no effects on bodyweight or food intake at any dose. The number of litters evaluated included 30 in the control group, 13 in the low dose group, and 15 in the two highest dose groups. Statistical analysis of fetal data included analysis of variance (ANOVA) for weight effects, the Kruskal-Wallis test for parameters such as litter size and percent alive/litter, and Fisher's exact test for malformations. For examination of skeletal effects, half the fetuses were fixed in 80percent ethanol, macerated in 1.5percent KOH, and stained with alizarin red S. The other half of fetuses were fixed in Bouin's solution and examined for visceral effects. Statistically significant and dose-related reductions in fetal weight were observed in the two highest dose groups. The increased number of litters with skeletal or visceral malformations was statistically significant at the 20,000 ppm dose. A range of visceral malformations were observed including exencephaly and encephalocele. Rudimentary and extra cervical ribs were the skeletal effects observed at the greatest frequency at the 20,000 ppm dose. The authors concluded that methanol was a definite teratogen at 20,000 ppm, a developmental toxicant (decreased fetal weight) and possible teratogen (numerical elevation of some

malformations) at 10,000 ppm, with a fetal no effect level of 5,000 ppm. A maternal [no observed adverse effect level] NOAEL of 10,000 ppm was noted by the Expert Panel.

Rogers et al. (1993) examined the sensitivity of Crl:CD-1 mice to the developmental toxicity of inhaled methanol (Table 7.3-B). In the original 3 block design, groups of mice were exposed to 1 of 4 doses of methanol vapors (Fisher Scientific Optima grade, ≥99.9percent purity) for 7 hours per day on gd 6 –15. The nominal doses and numbers of mice per dose (in parentheses) were air-exposed control *(114)*, 1,000 *(40)*, 2,000 *(80)*, 5,000 *(79)*, and 15,000 *(44)* ppm. A final block of mice was added to fill in intermediate concentrations of 7,500 *(30)*, and 10,000 *(30)* ppm. During the 7-hour inhalation exposure period, treated and air exposed mice were deprived of food but had access to water. An additional set of 88 controls were not handled (remained in their home cage) and fed *ad libitum*. Another group of 30 control mice remained in their home cage and were food deprived for 7 hours per day on gd 6 –15. Approximately 3 pregnant mice per block/treatment group were killed following exposure on gd 6, 10, or 15 and their blood was collected for plasma methanol analyses by GC. The mean plasma methanol concentrations averaged for the 3 gestational days were 1.6, 97, 537, 1,650, 3,178, 4,204, and 7,330 mg/L in the control to high-dose groups, respectively. Methanol plasma concentrations were dose-related, did not appear to reach saturation, and were not consistently affected by gestation day or previous days of exposure. Analysis of plasma methanol levels was conducted in a few non-pregnant mice and there appeared to be no differences compared to pregnant mice. Rogers et al. *(96)* noted that plasma levels at a given methanol concentration were lower in non-pregnant rats exposed through a similar protocol by Nelson et al. (1985).

Following sacrifice of dams on gd 17, Rogers et al. (1993) compared developmental effects in treated groups to effects in the chamber air-exposed control group. Dams and litters were considered the statistical unit and the numbers evaluated are listed under Table 7.3-B. Statistical analysis included the General Linear Models procedure and multiple T-test of least squares method for continuous variables and the Fisher's exact test for dichotomous variables. The chamber air-exposed control dams gained significantly less weight than both types of cage controls. Methanol exposure did not produce overt intoxication or further reduce weight gain in dams. There was a dose related and statistically significant decrease in the number of live pups per litter in groups exposed to methanol vapor doses of 7,500 ppm and higher; there was also a dose-related increase in females with fully resorbed litters at 10,000 ppm and higher. Fetal bodyweights were significantly reduced at 10,000 ppm and higher. The incidence of cleft palate was increased at doses of 5,000 ppm and greater. The percent incidence/litter of exencephaly was significantly increased at the 5,000, 10,000 and 15,000 ppm doses (not statistically significant at 7,500 ppm). Only fetuses from the 1,000, 2,000, 5,000, and 15,000 ppm groups were examined for either skeletal malformations or visceral defects. Skeletal effects were examined in half the fetuses that were fixed in 70percent ethanol, macerated with 1percent KOH, and stained with Alizarin red S. Visceral effects were examined in the other half of fetuses that were fixed in Bouin's solution. Delayed

ossification effects were commonly observed at the 15,000 ppm dose whereas several skeletal anomalies were seen at doses of 5,000 ppm and higher. The lowest dose at which an effect (cervical ribs) was observed was 2,000 ppm. Increased cervical ribs at 2,000 ppm was statistically significant in a pairwise comparison and showed a dose-response relationship with higher doses.

In this same study by Rogers et al. (1993), additional pregnant mice were exposed to methanol by the oral route to determine comparability of effects between exposure routes (Table 7.3-C). On gd 6 –15, 20 mice were gavaged with methanol twice daily at a dose of 2,000 mg/kg for a total dose of 4,000 mg/kg/day and 8 control pregnant mice were gavaged twice daily with water. The dose was selected to produce blood methanol levels observed in the inhalation study at the higher doses. Twice daily gavage doses of 2,000 mg/kg methanol (8 mice) on gd 6 –15 gave a pattern of response similar to that seen in the mouse group exposed to 10,000 ppm by inhalation. Mean maternal blood methanol levels 1 hour following the second daily exposure (3,856 mg/L) were slightly lower than blood levels in dams inhaling 10,000 ppm methanol in a previous experiment (4,204 mg/L). Fetal effects in the treated group included decreased fetal weight, increased resorptions, decreased live fetuses, and an increased incidence of fetuses/litter with cleft palate or exencephaly.

The Japanese New Energy Development Organization (NEDO, 1987) sponsored a study to evaluate the effects of prenatal exposure on prenatal and postnatal endpoints in Crl:CD Sprague-Dawley rats. Rats were randomly assigned to groups (n = 36/group) that were exposed to 0, 200, 1,000, or 5,000 ppm methanol vapors (reagent grade, stated to have <1 ppm vinyl chloride monomer and <3 ppm formaldehyde) on gd 7−17 for an average of 22.7 hours/day. The low dose in the study was selected because it is the ACGIH TLV, while higher doses were based upon observations in other studies sponsored by this group. Chamber concentrations of methanol were monitored and reported. Data were analyzed by t-test, Mann-Whitney U-test, Fisher's exact test and/or Armitage's χ^2-test.

In the assessment of prenatal development, a total of 19−24 dams/group were sacrificed on gd 20 and examined for implantation sites and number of corpora lutea. Fetuses were assessed for viability, sexed, weighed, and examined for external malformations. Half the fetuses were fixed in Bouin's solution and examined for visceral malformations. Skeletons from the remaining fetuses were stained with alizarin Red S and examined. Dams in the 5,000 ppm group experienced a reduction in bodyweight gain and food and water intake (statistical significance not reported) during the first 7 days of methanol exposure; 1 died on gd 19 and another was sacrificed in extremis on gd 18. Significant fetal effects were only observed at 5,000 ppm and included increased late resorptions, reduced numbers of live fetuses, decreased fetal weight, and increased numbers of litters containing fetuses with malformations, variations, and delayed ossification. Malformations noted were ventricular septal defect, while variations were noted in the thymus, vertebrae, and ribs (including cervical ribs).

Twelve dams/group were allowed to deliver and nurse their litters. The dams were sacrificed when pups were weaned and examined for implantation sites. Statistically significant effects noted in the 5,000 ppm group included prolonged gestation period (21.9±0.3 vs. 22.6±0.5 days in control and treated group), reduced post-implantation embryo survival (96.3±4.2percent vs. 86.2±16.2percent), and number of live pups/litter (15.2±1.6 vs. 12.6±2.5).

Youssef et al. (1997) conducted a study to determine toxicity of methanol in rats following oral administration at a single time point. On gd 10, 10 –12 Crl: Long-Evans rats were gavaged with methanol, HPLC grade, at 1.3, 2.6, or 5.2 mL/kg bw. The doses were selected according to guidelines for segment II studies that require one maternally toxic dose equal to 40percent of the LD_{50}. The rats were first gavaged with mineral oil to prevent gastric irritation. A control group of 9 rats was not gavaged and a control group of 4 rats was gavaged with mineral oil. Because no differences were found between the two control groups, data were combined into a single control group. Dams were sacrificed and necropsied on gd 20 and 10 –13 dams and fetuses per group were examined. Statistical analysis for fetal anomalies and variations included ANOVA, the Fischer PLSD exact test, and determination of dose-response relationships. Both the individual fetus and litter were considered statistical units. Signs of maternal toxicity were limited to the high dose group and included significantly decreased bodyweight gain and food intake. There were no signs of intoxication and a histological evaluation of tissues in two dams/group revealed no effects on liver, spleen, heart, lungs, and kidneys. Fetuses were examined grossly and the heads and skeleton were examined for malformations according to the Dawson method. Methanol exposure did not increase prenatal fetal mortality. Bodyweights of fetuses were significantly reduced in all treatment groups, but the response was not dose-related. The numbers of fetuses with anomalies or variations was significantly increased at all doses. Dose related anomalies included undescended testes and eye defects (exophthalmia and anophthalmia) that reached statistical significance in fetuses and litters of the high dose group. Other fetal effects that appeared to be dose related included facial hemorrhage, and dilated renal pelves. Authors noted that in contrast to previous rodent studies, exencephaly was not observed. According to authors, possible reasons for this discrepancy include differences in day of dosing, dose level, route of administration, or interspecies effect.

An *in vitro* study not available to the panel was conducted to determine if methanol could alter methylation of mouse embryonal (GD 8) DNA (Huang et al., 2001). Studies showed that culturing cells in methanol increased methylation of DNA at 4 mg/mL, but not at 8 mg/mL. The authors hypothesized that the lack of effect at the higher concentration might be due to embryo growth retardation. This study further showed that methanol exposure did not alter overall mouse embryonic protein levels or synthesis, but was specifically incorporated into lifestage-specific embryonal proteins. The authors noted that the concentrations used in the study correlated with peak serum methanol concentrations found in pregnant mice following inhalation exposures to 10,000 and

15,000 ppm methanol for 7 hours. This study provides further evidence that methanol could adversely affect embryo development at high concentrations.

References

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