Safety Assessment of Methyl Alcohol as Used in Cosmetics

Status:	Re-Review for Panel Consideration
Release Date:	November 10, 2022
Panel Meeting Date:	December 5-6, 2022

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Preethi Raj, Senior Scientific Analyst/Writer, CIR.

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Preethi S. Raj, M.Sc., Senior Scientific Writer/Analyst, CIR
Date: November 10, 2022
Subject: Re-Review of the Safety Assessment of Methyl Alcohol

The Expert Panel for Cosmetic Ingredient Safety (Panel) first published a review of the safety of Methyl Alcohol in 2001 (*originalreport_MethylAlcohol_122022*), with the conclusion that Methyl Alcohol is safe as used to denature alcohol used in cosmetic products.

Because it has been at least 15 years since the final report was published, in accordance with Cosmetic Ingredient Review (CIR) Procedures, the Panel should consider whether the safety assessment of Methyl Alcohol should be re-opened. An exhaustive search of the world's literature was performed for studies dated 1996 forward. Most of the toxicological and dermal irritation and sensitization data that was found is from a European Chemicals Agency (ECHA) dossier and a 2004 Screening Information Dataset (SIDS) Initial Assessment Report on Methyl Alcohol. Of note, subchronic oral and chronic inhalation studies in rats, mice, and monkeys, long-term exposure (18 mo up to a lifetime) carcinogenicity studies in mice and rats, as well as a guinea pig maximization test exhibiting weak, but negligible, sensitizing potential (50% Methyl Alcohol during induction and 100% during challenge) were found. An historical overview, comparison of original and new use data, and the search strategy used are enclosed herein (*newdata_MethylAlcohol_122022*).

Also included for your review is a table of current and historical use data (*usetable_MethylAlcohol_122022*). The number of reported uses has remained constant since the 2001 review. In 2022, FDA VCRP data indicate that Methyl Alcohol has 3 reported uses, while 4 uses were reported in 2001. The maximum use concentration for this ingredient appears to have decreased. Because concentration of use data were not reported to the FDA at the time of the 2001 report, 1984 data were used, which indicated that the reported concentration of use was 0.1 - 5%. In 2022, the maximum reported concentration of use is 0.15% in hair dyes and colors.

If upon review of the new studies and updated use data the Panel determines that a re-review is warranted, a Draft Amended Report will be presented at an upcoming meeting.

Re-Review - Methyl Alcohol - History and New Data

(Preethi Raj – December 2022 meeting)

Ingredients (1)	Citation	Conclusion	Use - New Data	Use - Historical Data	Notes
Methyl Alcohol	IJT 20(S1):57-85, 2001	safe as used to denature alcohol used in cosmetic products	frequency of use (2022): 3 uses conc of use (2021): 0.005 – 0.15%	frequency of use (1998): 4 uses conc of use (1984): 0.1 - 5%	Number of uses have remained the same: • Previously in 4 rinse-offs (other bath preps) • 2022, in 3 leave-ons Concentration of use has decreased from at up to 5% (use category unknown) to up to 0.15% in hair dyes and colors

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
Chemistry – ADME			
https://echa.europa.eu/registration-dossier/-/registered-dossier/15569/7/2/2/?documentUUID=b81d4578-4138-4a9a-b59d-af0dcb55b9ea	Chemistry properties	Appearance, melting/freezing point, pH, density	no
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/2/2/?documentUUID=91c77589-7f88-41b8-89e7-716ff6c5eaa8	ADME, oral	Male and female macaca fascicularis monkeys (3-4/group) received a single dose of 3000 mg/kg Methyl Alcohol, via gavage. After a latent period of 8 – 12 h, the monkeys that received Methyl Alcohol exhibited a syndrome common to primates followed by metabolic acidosis, coma, and death.	Not in original report
https://echa.europa.eu/registration-dossier/-/registered-dossier/15569/7/2/2	ADME, inhalation	OECD 471. Female CD-1 mice (8/group) were exposed to 3.3, 6.7, and 13 mg/l undiluted Methyl Alcohol vapor for 8 h (corresponding to 2500 – 10,000 ppm). Blood Methyl Alcohol concentrations were approximately 500 – 3000 mg/l at 1 h and 500 – 4000 mg/l at 6 h after exposure.	Not in original report
https://echa.europa.eu/registration-dossier/-/registered-dossier/15569/7/2/2/?documentUUID=b81d4578-4138-4a9a-b59d-af0dcb55b9ea	ADME, inhalation	OECD 471. Female Sprague-Dawley rats (4/group) were exposed to 1.3, 6.7, 13, 20, 26.6 mg/l undiluted Methyl Alcohol vapor for 8 h (corresponding to 1000 – 20,000 ppm). Blood Methyl Alcohol concentrations were approximately 100 – 4000 mg/l after exposure	Not in original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/2/2/?documentUUID=e3be3a56-8fe4-46ce-853e-4e7416d43575	ADME, inhalation	Non-pregnant Long-Evans rats were exposed to undiluted Methyl Alcohol vapor (5.98 mg/l) for 3 consecutive days for 6 h/d . Pregnant rats were exposed to undiluted Methyl Alcohol vapors from gestation day 6 to postnatal day 21 and Methyl Alcohol blood levels in dams and offspring were determined. During pregnancy and lactation, blood levels in dams were in the range of 0.55 ±0.07 and 0.56 ± 0.09 mg/ml, respectively. Methyl Alcohol levels in the offspring were twice that of dams, at 1.26 ± 0.23 mg/ml, which gradually decreased to that of the dams after 52 postnatal days.	Not in original report

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
https://echa.europa.eu/registration-dossier/-/registered-dossier/15569/7/2/2/?documentUUID=b76f6583-ca03-40ed-8e86-7a3fbba3ec7a	ADME, inhalation	Male macaca mulatta monkeys (3/group) were exposed to 0.06, 0.27, 1.60, or 2.66 mg/l undiluted Methyl Alcohol vapor for 6 h (corresponding to 50 – 2000 ppm), to compare to toxicokinetics in human and rat models. Blood Methyl Alcohol concentrations post-exposure were similar across species below 1.6 mg/l, and an increase in blood concentrations became non-linear for rats and monkeys, while remaining linear for humans.	Not in original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/2/2/?documentUUID=0c9178af-4ac4-42f0-bd89-d50ab90bfe32	ADME, inhalation	Female macaca fascicularis monkeys (1 animal/dose) were exposed to 0.01, 0.06, 0.27, or 1.2 mg/l undiluted Methyl Alcohol vapor for 2 h. Methyl Alcohol blood concentrations increased during exposure and declined rapidly after completion of the exposure; levels were undetectable between 8 – 10.5 h after exposure	Not in original report
https://echa.europa.eu/registration-dossier/-/registered-dossier/15569/7/2/2/?documentUUID=9922d618-510b-4ae4-912c-6b448d981d6a	ADME, inhalation	Female macaca fascicularis monkeys (9-12/group) were exposed to 0, 0.27, 0.8, or 2.4 mg/l undiluted Methyl Alcohol vapor (corresponding to 0, 200, 600, and 1800 ppm) for 2.5 h before breeding, during breeding, and during pregnancy. No significant differences in blood concentrations of Methyl Alcohol were observed between non-pregnant and pregnant monkeys across dosage groups when measured 0.5 h post exposure at each stage. At 1800 ppm, after 5 h elimination, the residual Methyl Alcohol level was near baseline.	Not in original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/11/6/?documentUUID=0c9dd9c8-1a2d-40da-ab9d-473248bd67fb	ADME, dermal, human	12 subjects had Methyl Alcohol applied dermally to one hand for a duration of 0-16 min, for a total of 65 sessions. Compared to the pre-exposure Methyl Alcohol blood concentration of 1.7 mg/l, the maximum mean Methyl Alcohol concentration in blood reached 1.9 h after the last exposure. Mean derived absorption rate: 8.1 ± 3.7 mg/cm^2/h	Not in original report
Non-Cosmetic			
https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=173.250	CFR for use as secondary direct food additive	21CFR173.250 Sec. 173.250 Methyl alcohol residues. Methyl alcohol may be present in the following foods under the conditions specified: (a) In spice oleoresins as a residue from the extraction of spice, at a level not to exceed 50 parts per million. (b) In hops extract as a residue from the extraction of hops, at a level not to exceed 2.2 percent by weight; Provided, That: (1) The hops extract is added to the wort before or during cooking in the manufacture of beer.	CFR not mentioned in the original report

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
		(2) The label of the hops extract specifies the presence of methyl alcohol and provides for the use of the hops extract only as prescribed by paragraph (b)(1) of this section.	
https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=176.180	CFR for use in food packaging	21CFR175.105; 21CFR176.200; 21CFR176.210; 21CFR176.80 – state that methyl alcohol can be safely used as a component of adhesives, defoaming agents, paper and paperboard products that come in contact with food	This use/CFR is not mentioned in the original report
Toxicity			
OECD SIDS Initial Assessment Profile: Methanol (Methyl Alcohol;2004) – page 16: https://hpvchemicals.oecd.org/ui/handler.axd?id=f6aca735-7365-4578-986c-5a3cd5027555	Acute dermal tox	Rats survived occlusive application of 35,000 mg/kg bw Methyl Alcohol – deaths were reported at 45,000 mg/kg bw	Not in original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/3/4	Acute dermal tox	Rabbits; $LD_{50:}$ 20 ml/kg (further details not provided)	no
OECD SIDS Initial Assessment Profile: Methanol (Methyl Alcohol;2004) – page 16: https://hpvchemicals.oecd.org/ui/handler.axd?id=f6aca735-7365-4578-986c-5a3cd5027555	Acute dermal tox	A dermal LD_{50} of ~ 17,000 mg/kg bw was determined in rabbits (; no further details provided)	Not in original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/2/2/?documentUUID=9a8be4cb-db91-4f4a-a359-f0fd61299b40	Acute oral tox	Catalase wildtype and catalase deficient male mice (6/group) were dosed with 2000, 4000, 5000, 6000, 7000, 8000, 9000, or 10,000 mg/kg bw Methyl Alcohol in water, via gavage. Mice with a catalase deficiency showed a somewhat lower LD_{50} oral value than those with a normal catalase level (LD_{50} value not provided).	Not in original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/3/2	Acute oral tox	OECD TG 401; rats (10/group); 2528 mg/kg bw, diluted to 50% in water $LD_{50} \geq$ 2528 mg/kg bw	no
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/3/2/?documentUUID=ecdd8be5-c0d1-468c-8042-e2202b284978	Acute oral tox	Male/female Sprague-Dawley rats (10/group); 551-1285, 808-1885, 1187-2769 mg/kg bw (corresponding to 15-35% aqueous solution) LD_{50} > 1187-2769 mg/kg bw	no
OECD SIDS Initial Assessment Profile: Methanol (Methyl Alcohol;2004)- page 16: https://hpvchemicals.oecd.org/ui/handler.axd?id=f6aca735-7365-4578-986c-5a3cd5027555	Acute oral tox	In rats, mice, rabbits, and dogs the LD_{50} values after single oral administration range from 5600 to 14,400 mg/kg bw (no further details provided)	Not in original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/3/2/?documentUUID=b1a7c6d7-ada2-4bac-9959-959ff746ff07 *AND* *OECD SIDS Initial Assessment Profile: Methanol(Methyl Alcohol;2004)- page 16*	Acute oral tox	8 Rhesus monkeys were orally dosed with 1000 -2000 mg/kg bw Methyl Alcohol, which did not lead to mortality; animals which received 3000-8000 mg/kg bw Methyl Alcohol died within 2 d (see below re: bicarbonate supplementation). LD_{50}: 6000 mg/kg bw; 4 of the animals died, and 4 survived after bicarbonate supplementation	Not in original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/3/2/?documentUUID=469c9de1-6a7e-4a69-a2ab-e333a371b3bb *AND* *OECD SIDS Initial Assessment Profile: Methanol (Methyl Alcohol;2004)- page 17*	Acute oral tox	12 Rhesus monkeys were orally dosed with 500-4000, 5000, 6000, 7000, 8000 or 9000 mg/kg Methyl Alcohol, at 20-30% in water No evidence of marked acidosis was seen at up to 6000 mg/kg bw. One monkey dosed with 9000 mg/kg bw went blind; the effect lasted for 4 d. $LD_{50:}$ 7000 - 9000 mg/kg bw	no

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/6/2/?documentUUID=627edd29-f784-4fc6-a5ad-4c56885ea626	Short-term oral tox	7 male monkeys; 30% Methyl Alcohol in water (2340 mg/kg bw/d) was administered via gavage for 3 d. The received dose was lethal to all 7 animals after 7 d. LOAEL was determined to be 2340 mg/kg bw	Not in original report
OECD SIDS Initial Assessment Profile: Methanol(Methyl Alcohol;2004)- page 143: https://hpvchemicals.oecd.org/ui/handler.axd?id=f6aca735-7365-4578-986c-5a3cd5027555	Subchronic oral tox	Male and female Sprague-Dawley rats (30/group) received 0, 100, 500, or 2500 mg/kg bw Methyl Alcohol for 90 d. There were no histopathological effects; elevated levels of liver enzymes and liver weights and lower brain weights were found in high dose males and females. NOAEL was determined to be 500 mg/kg/d	No repeated dose oral tox in the original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/3/3/?documentUUID=4c2d890c-ba8c-4952-a132-19a8b84f1c8a	Acute inhalation tox	Male/female Sprague-Dawley rats (10/group); received 87.6, 115.9, 139.0, 150.9, or 151.1 mg/l undiluted, Methyl Alcohol vapor nose/head only for 4 h. At concentrations of 150.9 mg/l Methyl Alcohol, the body weight of male animals was reduced by 33%. No mortality occurred and no findings were made upon necropsy.	no
OECD SIDS Initial Assessment Profile: Methanol (Methyl Alcohol; 2004) – page 16: https://hpvchemicals.oecd.org/ui/handler.axd?id=f6aca735-7365-4578-986c-5a3cd5027555	Acute inhalation tox	LC_{50} values for Sprague-Dawley rats were 87.5 mg/l for 6 h and 128.2 mg/l for 4 h, respectively (no further details provided). Clinical signs of toxicity were aqueous secretion of eyes and nose, labored breathing, staggering, apathy, and narcosis	no
OECD SIDS Initial Assessment Profile: Methanol (Methyl Alcohol;2004) – page 143: https://hpvchemicals.oecd.org/ui/handler.axd?id=f6aca735-7365-4578-986c-5a3cd5027555	Short-term inhalation tox	Male and female Sprague-Dawley rats (5/group) were exposed to 0, 0.663, 2.65, or 6.63 mg/l Methyl Alcohol for 6 h/d, 5 d/wk for 4 wk. No deaths occurred. Only mucoid nasal discharge appeared to be dose related. NOAEL: 6.63 mg/l	no
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/6/3/?documentUUID=cdf53a02-7036-44ab-ba9d-a3fe34d65062	Short-term inhalation tox	Male Sprague-Dawley rats were exposed to 0.265 (200 ppm), 2.65 (2000 ppm), or 13.3 (10,000 ppm) mg/l undiluted Methyl Alcohol vapor for up to 6 wk, at 8 h/d and 5 d/wk. The NOAEC/LOAEC was determined to be 2.65 mg/l due to the significant increase in circulating luteinizing hormone after 6 wk of exposure	Not in original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/6/3/?documentUUID=15698667-3314-4ead-b72b-10099f11f462	Short-term inhalation tox	Male Sprague-Dawley rats (9/group) were exposed to 0.26 mg/l undiluted Methyl Alcohol vapor for up to 6 wk at 8 h/d and 5 d/wk. NOAEC = 0.26 mg/l for testicular production of testosterone. No other effects were observed.	Not in original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/6/3/?documentUUID=b4b49c0b-84d2-4488-9f01-1be88b3fa11b	Short-term inhalation tox	Male Sprague-Dawley rats (11-12/group; 8 for highest dose) were exposed to 0.066, 0.266, or 1.06 mg/l undiluted Methyl Alcohol vapor for 13 wk, at 20 h/d and 7 d/wk. NOAEC = 1.06 mg/l, based on testicular histopathology	Not in original report
OECD SIDS Initial Assessment Profile: Methanol (Methyl Alcohol;2004) – page 144-145: https://hpvchemicals.oecd.org/ui/handler.axd?id=f6aca735-7365-4578-986c-5a3cd5027555	Short-term inhalation tox	Macaca fascicularis monkeys were exposed for 21 h/d to 13, 9.1, 6.5, or 3.9 mg/l Methyl Alcohol for 15 d. Animals at the top dose showed lethargy, and 3 fell into coma and died.	no

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
		Animals in the 9.1 mg/l group were killed because of suffering. LOAEL: 3.9 mg/l	
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/6/3/?documentUUID=40873907-cf33-4e66-9d9f-d22f30d6bda0	Chronic inhalation tox	OECD TG 453. Male/female B6C3F1 mice (30/group) were exposed 19.8 h/d, whole body, to 0.013, 0.13, or 1.3 mg/l Methyl Alcohol vapor for 12 mo. NOEC = 1.3 mg/l	Chronic inhalation tox not in original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/6/3/?documentUUID=81c7cd51-d717-4eec-a3e5-092b18c48df9	Chronic inhalation tox	Male/female Fischer rats (20/group) were exposed 20 h/d whole body to 0.013, 0.13, or 1.3 mg/l Methyl Alcohol vapor for 12 mo. No significant effects were observed. LOAEC = 1.3 mg/l	Chronic inhalation tox not in original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/6/3	Chronic inhalation tox	Macaca fascicularis monkeys were exposed, whole body, to Methyl Alcohol vapor, 21 h/d at concentrations of 0.013, 0.13, or 1.3 mg/l for 7 mo (2 animals), 19 mo (3 monkeys), or 29 mo (3 monkeys). No critical effects were observed	Chronic inhalation tox not in original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/6/3/?documentUUID=28e989fc-4d79-478d-9945-056f4e82c915	Chronic inhalation tox	Macaca fascicularis monkeys (2-4/group) were exposed, whole body, to 1.3, 2.7, 4, 5.3, or 6.7 mg/l undiluted Methyl Alcohol vapor for 7 mo. No critical effects were observed.	Chronic inhalation tox not in original report
Genotoxicity			
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/7/2	Genotoxicity; in vitro	*Aspergillus nidulans* was treated with undiluted Methyl Alcohol at concentrations of 5.2, 5.6, 6, or 7% (v/v). There was a concentration-related increase in non-disjunctions with a maximum of 3%. Considered positive for genotoxicity.	no data on *Aspergillus* in original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/7/2/?documentUUID=afd2e313-49d1-4412-8605-9e2ffb8946ba	Genotoxicity; in vitro	DNA damage/repair study using *Escherichia coli* WP2, WP67, CM871. 40 mg Methyl Alcohol/well, in the presence and absence of metabolic activation. Negative for genotoxicity. MIC = 120 g/l	no
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/7/2/?documentUUID=1913d04f-b765-4b04-b617-c1f8c8099c99	Genotoxicity, in vitro	OECD TG 471. Bacterial reverse mutation assay TA97 and TA102, treated with up to 7.5 mg/plate, in the presence of metabolic activation. Slight positive trend seen for TA 102, but test-article related increase in mutation frequency did not fulfill the criteria for mutagenic actiivity	No bacterial reverse mutation assay in original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/7/2/?documentUUID=c343e70b-b2e6-4341-97b9-4e514e49bc8a	Genotoxicity, in vitro	OECD TG 471. Bacterial reverse mutation assay. *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 15538 were tested with up to 5000 µg/plate Methyl Alcohol, in the presence and absence of metabolic activation; not genotoxic	No bacterial reverse mutation assay in original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/7/2/?documentUUID=897f294a-5c50-4691-a410-790d34fe9858	Genotoxicity, in vitro	Gene mutation assay. Mouse lymphoma L5178Y cells were tested with 7.9 mg/ml Methyl Alcohol in the presence of 10 – 15 µl/ml metabolic activation agent (S9). There was a significant increase in mutation frequency (no further details provided).	no
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/7/2/?documentUUID=3e587acd-1949-40ea-ad4e-63dd60779642	Genotoxicity, in vitro	Mammalian cell micronucleus test. Chinese hamster lung fibroblasts (V79) were tested with concentrations of up to 50	no

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
		µl/ml Methyl Alcohol, in Eagle's medium for 48 h (acetone for positive controls). Was deemed not genotoxic in the absence of metabolic activation.	
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/7/2/?documentUUID=e49982e1-8d06-4aaa-9bd8-0ef6556af279	Genotoxicity, in vitro	OECD TG 476. Mammalian cell gene mutation assay. Chinese hamster lung (V79) fibroblasts were tested with 15.8, 31.7, 47.4, or 63.3 mg/ml Methyl Alcohol (in Eagle's medium), with or without metabolic activation.; not genotoxic	no
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/7/3/?documentUUID=0ebb4b30-db07-4ae9-bf68-77528807f10b	Genotoxicity, in vivo, inhalation	Mammalian chromosome aberration test. Male mice (5/group) received whole body undiluted Methyl Alcohol vapor for 6 h/d (1.04 or 5.3 g/l) for 5 d. Blood was drawn and primary cultures of lung cells were taken following the last exposure. There were no indications of genotoxicity.	no
Carcinogenicity			
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/8/?documentUUID=25054166-9b92-4636-9362-faf2c5a6a622	Oral, lifetime exposure	Male Swiss mice received 550, 970, or 1800 mg/kg/d Methyl Alcohol Female Swiss mice received 560, 1000, or 2100 mg/kg/d Methyl Alcohol Both for 6 d/wk, for a lifetime. Increased incidence of liver parenchymal cell necrosis and malignant lymphoma was observed. LOAEL: 1800 -2100 mg/kg bw/d, based on overall lack of negative effects	No carcinogenicity data in the original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/8/?documentUUID=7aa421a3-a112-4992-a571-7dcd77b02527	Inhalation, 18 mo	Male and female B6C3F1 mice (52-53/group) were exposed to undiluted, whole body, inhalation vapor at 0.013, 0.13, 1.3 mg/l of Methyl Alcohol for 18 mo, 19 h/d. NOAEC: ≥ 1.3 mg/l; No treatment-related effects were observed	No carcinogenicity data in the original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/8/?documentUUID=27c7a38f-9a19-4512-b42b-9bf2359bf57c	Inhalation, 24 mo	Male and female Fischer 344 rats (52/group) were exposed 20 h/d, whole body, to undiluted Methyl Alcohol vapor for 24 mo, at concentrations of 0.013, 0.13, or 1.3 mg/l. No critical effects were observed. NOAEC ≥ 1.3 mg/l	No carcinogenicity data in the original report
Developmental and Reproductive Toxicity Studies			
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/2/2/?documentUUID=e1ffe90d-164c-4930-9130-b5516f72e2c4	DART, in vivo	Impregnated female mice (2-16/group) were killed on day 9 of gestation and embryos containing 7 or 8 somite pairs were explanted and cultured for 24 h in a medium of 0.9% saline or 4 mg/ml Methyl Alcohol. Embryos exposed to Methyl Alcohol expressed dysmorphogenesis evidenced by decreases in anterior neuropore closure, turning, and somite development. Embryos expressing human catalase were protected from embryopathies, while those not expressing catalase were more susceptible to embryopathies, evidenced by decreased anterior neuropore closure (100%), yolk-sac diameter (13%), and crown-rump length (37%), compared to wildtype embryos.	no

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
Dermal Irritation/Sensitization Studies			
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/4/2	Skin irritation	2 Vienna white rabbits; undiluted Methyl Alcohol was dermally applied for 1, 5, or 15 min or 20 h. No signs of irritation (erythema, edema) were apparent after 24, 48, and 72 h, and after 6 and 8 d	no
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/5/2 *AND* *OECD SIDS Initial Assessment Profile: Methanol (Methyl Alcohol;2004) – page 135:* *https://hpvchemicals.oecd.org/ui/handler.axd?id=f6aca735-7365-4578-986c-5a3cd5027555*	Dermal sensitization	OECD TG 406: guinea pig maximization test (12 test and 5 control animals). Four induction applications were made, including two intradermal injections of 50% Methyl Alcohol and FCA and two, 48-h occlusive epicutaneous applications of 100% Methyl Alcohol. A 24-h challenge application of 100 % Methyl Alcohol was made 34 d after the first intradermal injection and the site was evaluated for up to 72 h. One animal exhibited slight erythema (score 1) 24 and 48 h after challenge, suggesting weak sensitizing potential, which was not considered significant.	Guinea pig maximization test not in original report
Ocular Irritation Studies			
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15569/7/4/3	Ocular irritation	2 rabbits; undiluted Methyl Alcohol (0.05 ml) was applied to the eyes and not rinsed for an unspecified time interval. After 1 h, mild erythema and cornea opacity as well as moderate edema were observed and the effects were assessed as mild after 24 h. The animals exhibited no symptoms after 8 d.	No rabbit ocular irritation in the original report

Abbreviations: FCA – Freund's complete adjuvant; LOAEC-/LOAEL - lowest-observed-adverse-effect-level; MIC - minimum inhibitory concentration; NOAEC – no-observed-adverse-effect-concentration; NOEC- no-observed-effect-concentration; NOAEL – no- observed- adverse-effect-level; NOEL- no-observed-effect-level; OECD – Organisation for Economic Cooperation and Development; SIDS – Screening Information Dataset; TG – test guideline

PubMed Search (from 1996 to present)

[hits/hits that were useful]- as of 10/28/2022

(((((((Methyl alcohol) OR (methanol)) OR (carbinol)) OR (methyl hydroxide)) OR (methylol)) OR (wood alcohol)) OR (67-56-1)) AND (cosmetic toxicity))
-AND (cosmetic toxicity)) – 133/0

General Web Search

Methyl alcohol; methanol; cosmetic safety – 27,600,00/6

Table 1. Current and historical frequency and concentration of use according to duration and exposure for Methyl Alcohol

	# of Uses		*Max Conc of Use (%)*					
	Methyl Alcohol							
	2022[1]	**1998[2]**	**2021[3]**	**1984[2]**				
Totals*	**3**	**4**	**0.005 – 0.15**	**0.1-5****				
Duration of Use								
Leave-On	*3*	*NR*	*0.005-0.006*	**				
Rinse-Off	*NR*	*NR*	*0.15*	**				
Diluted for (Bath) Use	*NR*	*4*	*NR*	**				
Exposure Type								
Eye Area	1	NR	NR	**				
Incidental Ingestion	NR	NR	NR	**				
Incidental Inhalation-Spray	1[b]	NR	0.006; 0.005[a]	**				
Incidental Inhalation-Powder	1[b]	NR	NR	**				
Dermal Contact	2	4	NR	**				
Deodorant (underarm)	NR	NR	NR	**				
Hair - Non-Coloring	1	NR	0.005 – 0.006	**				
Hair-Coloring	NR	NR	0.15	**				
Nail	NR	NR	NR	**				
Mucous Membrane	NR	4	NR	**				
Baby Products	NR	NR	NR	**				

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
at the time of the **2001 safety assessment, concentration of use data were not reported by the FDA; 1984 data were presented
[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.
[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories
NR – not reported

References

1. U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition (CFSAN). 2022. Voluntary Cosmetic Registration Program - Frequency of Use of Cosmetic Ingredients (VCRP). (Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 4, 2022; received January 11, 2022.)

2. Lanigan S. Final report on the safety assessment of Methyl Alcohol. *Int J Toxicol.* 2001;20 Suppl 1:57-85.

3. Personal Care Products Council. 2021. Concentration of Use by FDA Product Category: Methyl Alcohol. (Unpublished data submitted by Personal Care Products Council on January 12, 2022.)

Final Report on the Safety Assessment of Methyl Alcohol[1]

Methyl Alcohol is an aliphatic alcohol with use in a few cosmetic formulations as a solvent and denaturant. Concentrations up to 5% are typically used to denature ethyl alcohol in cosmetic products. Methyl Alcohol is readily absorbed through the skin and from the gastrointestinal and respiratory tracts, is distributed throughout all organs and tissues (in direct relation to the body's water distribution), and is eliminated primarily via the lungs. Undiluted Methyl Alcohol is an ocular and skin irritant. Inhalation studies showed a no-effect level for maternal damage of 10,000 ppm and for teratogenic effects of 5000 ppm. Overall, Methyl Alcohol is not considered mutagenic. Carcinogenicity data were unavailable. The toxicity of Methyl Alcohol in humans results from the metabolism of the alcohol to formate and formic acid through a formaldehyde intermediate. Formate accumulation causes metabolic acidosis and inhibits cellular respiration. Methyl Alcohol toxicity is time and concentration dependent, and its toxic effect is competitively inhibited with ethyl alcohol. Because of the moderating effect of ethyl alcohol, it was concluded that Methyl Alcohol is safe as used to denature ethyl alcohol used in cosmetic products. No conclusion was reached regarding any other use of Methyl Alcohol.

INTRODUCTION

Methyl Alcohol is an aliphatic alcohol that serves as a solvent and denaturant in cosmetic formulations. Methyl Alcohol is metabolized to formaldehyde and formic acid, both of which were previously reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel:

> Formaldehyde in cosmetic products is safe to the great majority of consumers. The Panel believes that because of skin sensitivity of some individuals to this agent, the formulation and manufacture of a cosmetic product should be such as to ensure use at the minimal effective concentration of formaldehyde, not to exceed 0.2 percent measured as free formaldehyde. It cannot be concluded that formaldehyde is safe in cosmetic products intended to be aerosolized (Elder 1984). Formic Acid is safe as used as a pH adjuster with a 64-ppm limit for the free acid (Formic Acid also dissociates to formate) (CIR 1995).

Methyl Alcohol is readily absorbed through the skin and from the gastrointestinal and respiratory tracts, and distribution is uniform throughout all organs and tissues in direct proportion to their water content, regardless of the route of exposure. The acute toxicity of Methyl Alcohol has been well established and has been documented extensively in the published literature.

CHEMISTRY

Definition and Structure

Methyl Alcohol (CAS No. 67-56-1) is the aliphatic alcohol that conforms to the formula CH_3OH. Methyl Alcohol is also known as Methanol (Wenninger, Canterbery, McEwen 2000; Registry of Toxic Effects of Chemical Substances [RTECS] 1996); Carbinol; Colonial Spirit; Columbian Spirit; Methylol; Methyl Hydrate; Methyl Hydroxide; Monohydroxymethane; Pyroxylic Spirit; Wood Alcohol; Wood Naphtha; Wood Spirit (Radian Corporation 1991; Acros Organics 1996; Fisher Scientific 1996; RTECS 1996); Eagle Spirit; Manhattan Spirit; Hastings Spirit; Lion d'Or; Methylated Spirit; and Acetone Alcohol (Bennett et al. 1953).

Chemical and Physical Properties

The chemical and physical properties of Methyl Alcohol are listed in Table 1.

Reactivity

In air, Methyl Alcohol exists in the vapor phase with a half-life of 17.8 days. Methyl Alcohol reacts with photochemically produced hydroxyl radicals to form formaldehyde. Methyl Alcohol can also react with nitrogen dioxide in polluted air to form methyl nitrite. Methyl Alcohol in water biodegrades into methane and carbon dioxide (Environmental Protection Agency [EPA] 1994a).

Undiluted Methyl Alcohol is stable under normal laboratory conditions, as are solutions of Methyl Alcohol in water, dimethyl sulfoxide (DMSO), 95% ethyl alcohol; and acetone (Radian Corp. 1991).

Methyl Alcohol is highly flammable (Radian Corp. 1991; EPA 1994a; National Academy of Sciences [NAS] 1995; Acros Organics 1996; Fisher Scientific 1996) and burns with an invisible flame in daylight. Its vapor can travel a considerable distance

Received 15 June 2000; accepted 2 November 2000.

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International Journal of Toxicology, 20(Suppl. 1):57–85, 2001

1071-7544/01 $12.00 + .00

TABLE 1
Chemical and physical properties of Methyl Alcohol

Property	Description	Reference
Appearance and physical state	Clear, colorless liquid	EPA 1994a; National Academy of Sciences (NAS) 1995; Acros Organics 1996; Fisher Scientific 1996
Molecular weight	~32 Da	Radian Corp. 1991; EPA 1994a; Acros Organics 1996; Fisher Scientific 1996; RTECS 1996
Melting point (°C)	(−)97.8–(−)98	Radian Corp. 1991; EPA 1994a; NAS 1995
Boiling point (°C)	64.6–65.15	Radian Corp. 1991; EPA 1994a; NAS 1995
Flash point (°C)	11–12; highly flammable	Radian Corp. 1991; EPA 1994a; NAS 1995; Fisher Scientific 1996
pH	Neutral	Acros Organics 1996; Fisher Scientific 1996
Vapor pressure	96 mm Hg at 20°C	NAS 1995
	100 mm Hg at 21.1°C and 237.87 mm Hg at 38°C	Radian Corp. 1991
	126 mm Hg at 25°C	EPA 1994a
Vapor density (air = 1)	1.1	NAS 1995; Acros Organics 1996; Fisher Scientific 1996
Evaporation rate (ether = 1)	5.2	Acros Organics 1996; Fisher Scientific 1996
Viscosity	0.006 P at 68°F	Acros Organics 1996; Fisher Scientific 1996
Solubility	Soluble in water, DMSO, 95% ethyl alcohol, acetone, and most organic solvents; miscible in chloroform, ketones, ether, and benzene	Radian Corp. 1991; EPA 1994a; NAS 1995
Odor	Slight alcohol odor; detected at >4 ppm	Radian Corp. 1991; EPA 1994a; NAS 1995; Acros Organics 1996; Fisher Scientific 1996
Half-life	17.8 days	EPA 1994a
Density	0.7915–0.796 g/ml	Radian Corp. 1991; EPA 1994a
K_{OC}	9	EPA 1994a
log K_{OW}	−0.77	EPA 1994a
Specific gravity (water = 1)	0.8	EPA 1994a; Acros Organics 1996; Fisher Scientific 1996
Autoignition temperature (°C)	383–463.89	Radian Corp. 1991; NAS 1995; Fisher Scientific 1996
Refractive index	1.3292 at 20°C	Radian Corp. 1991

to an ignition source and "flash back." Methanol-water mixtures will burn unless extremely dilute (NAS 1995).

Methyl Alcohol can react violently with acids, acid chlorides, acid anhydrides, strong oxidizing agents, reducing agents, and alkali metals (EPA 1994a; NAS 1995; Acros Organics 1996; Fisher Scientific 1996).

Method of Manufacture

Methyl Alcohol is a primary liquid petrochemical made from renewable and nonrenewable fossil fuels containing carbon and hydrogen. Methyl Alcohol is manufactured from synthesis gas, which is produced from steam-reformed natural gas and carbon dioxide. Methyl Alcohol is synthesized under pressure in a catalytic process and is purified by distillation (Methanex 1996).

Methyl Alcohol has historically been produced from the distillation of wood, and occurs naturally in wood and volcanic gases (EPA 1994b). The distillation products were then neutralized with lime (Browning 1965).

Methyl Alcohol is also obtained through the condensation of hydrogen with carbon dioxide or carbon monoxide (Von Burg 1994) at elevated temperature and pressure. This method of manufacture is most commonly used for commercial and industrial applications (Kavet and Nauss 1990).

Impurities

Natural Methyl Alcohol, produced by the distillation of hardwood, contained 1% to 2% of propyl and allyl alcohol, aldehyde,

methyl acetate, acetone, and other organic compounds. Synthetic Methyl Alcohol, the commercially available product, was "practically pure" (Browning 1965). Commercial Methyl Alcohol may contain traces (not quantified) of acetone, acetic acid, and aldehydes (Von Burg 1994).

USE

Cosmetic

Methyl Alcohol functions as a solvent and denaturant in cosmetic formulations (Wenninger, Canterbery, McEwen 2000). Data submitted to the Food and Drug Administration (FDA) in 1998 indicated that Methyl Alcohol was used in four cosmetic formulations in the category of "other bath preparations" (FDA 1998). Concentration of use data are no longer reported to the FDA, but Methyl Alcohol was used at concentrations of 0.1% to 5% in 1984 (FDA 1984).

International

In the European Union, Methyl Alcohol is used as a denaturant for ethyl alcohol and isopropyl alcohol (to render them unfit for consumption) in cosmetic product formulations. The maximum concentration of use is 5%, which is calculated as a percentage of the other alcohols. Otherwise, Methyl Alcohol is prohibited from use in cosmetic products (EEC Cosmetic Directive 1991).

Noncosmetic

Methyl Alcohol functions as a denaturant for ethyl alcohol, and is a starting material for the synthesis of chemicals such as formaldehyde, methyl-*t*-butyl ether, methacrylates, methylamines, methyl halides, and ethylene glycol (EPA 1994b; Von Burg 1994).

Methyl Alcohol is a widely used solvent in the agricultural and pharmaceutical industries. Methyl Alcohol is used in the extraction of agricultural products such as hops and spices (Rothschild 1990; EPA 1994b). It is an animal and vegetable oil extractant, and is an indirect food additive permitted to come into contact with foods for human consumption (Rothschild 1990; Radian Corp. 1991). Up to 50 ppm can be present in spice oleoresins and 2.2% (by weight) can be present in hops extract as residues from the extraction processes. In the case of the latter, Methyl Alcohol can be present only when the hops extract is added to the wort (liquid malt solution) before or during cooling in the manufacture of beer. The label of the extract must specify the use of hops only in this way, and declare the presence of Methyl Alcohol (Rothschild 1990).

Methyl Alcohol is present in all alcoholic beverages (Von Burg 1994). Imported brandy can contain a maximum concentration of 0.35% Methyl Alcohol. Above this concentration, the FDA will take legal action to remove human and animal feed products from the market; the concentration is established based on the unavoidability of poisonous or deleterious substances during processing or manufacture (FDA 1994).

Methyl Alcohol is available in the diet from fresh fruits and vegetables, or from fruit juices (average 140 mg/l). Fermented beverages contain up to 1.5 g/l Methyl Alcohol. Aspartame, a commonly used artificial sweetener, hydrolyzes to Methyl Alcohol in the gut such that 10% becomes free Methyl Alcohol that is available for absorption. The intake of a 12-oz beverages containing aspartame is roughly equivalent to the intake of 20 mg Methyl Alcohol.

The toxic metabolite of Methyl Alcohol, formate, is a natural ingredient in honey (2–200 mg/100 g), fruit syrups (65–163 mg/100 g), and roasted coffee (200–770 mg/100 g). Formate is also used as a preservative (Kavet and Nauss 1990).

Formaldehyde is a normal metabolite in mammalian systems, and occurs in air as a product of photooxidation of automobile exhaust, combustion processes, and incinerators. It has been detected in municipal and industrial effluents and is present in food either naturally (fresh and preserved fish seafood, honey, roasted foods, fruits and vegetables at up to 22.3 ppm) or as a result of its use as a food additive (packaging, preservation, cheese production). Formaldehyde is also used in animal feed technology to produce the rumen bypass of dietary proteins or to encapsulate lipids into protein shells. It binds to proteins, thus decreasing the protein solubility and deamination in the rumen; the bond between the protein and formaldehyde is broken in the lower part of the digestive tract, without liberation of undesirable compounds stored in edible animal products (Restani and Galli 1991).

Methyl Alcohol is used as a solvent in the manufacture of cholesterol, streptomycin, vitamins, hormones, and other pharmaceuticals (Radian Corp. 1991). Methyl Alcohol is a component of various pesticides, gasohol, gasoline additives, and antifreeze. It is used in solid fuel (e.g., Sterno), paint and varnish removers, embalming fluids, photocopier toners, celluloid, textile soap, wood stains, coated fabrics, shatterproof glass, paper coatings, waterproofing formulations, artificial leather, synthetic dyes, photographic film, and other products (Andrews et al. 1987; Radian Corp. 1991; EPA 1994b).

GENERAL BIOLOGY

Absorption, Metabolism, Distribution, and Excretion

Methyl Alcohol is readily absorbed from the gastrointestinal and respiratory tracts, and is absorbed percutaneously. The reported degree of absorption varied from 60% to 100%, and the recommended permeability coefficient for percutaneous transport of Methyl Alcohol was 1.6×10^{-3}cm h^{-1} (Von Burg 1994).

The logP of Methyl Alcohol was −0.68. The investigators concluded that in vivo skin absorption of volatile organic solvents at intermittent exposure was less than at continuous exposure; absorption was related to the volatility of the solvents (Boman and Maibach 1996). In another study (Dutkiewicz, Kończalik, and Karwacki 1980), 0.19 to 0.21 cm^3 of the alcohol was applied (for up to 60 minutes) using a micropipette onto a glass applicator, which was attached to the skin of the forearm

of six subjects. The mean rate of Methyl Alcohol absorption through human skin was 0.192 mg/cm^2/min.

Franz, Lehman, and Franz (1991) reported that Methyl Alcohol was readily absorbed through human skin, and the peak rate of absorption of a finite dose was reached within 30 minutes. The total absorption was low (2% of the dose, unspecified); most of the applied dose was lost by volatilization. Application of an infinite dose resulted in a steady-state rate of absorption of ~15 μl/cm^2/h. When Methyl Alcohol was applied to the skin for varying lengths of time, total absorption was linearly related to the contact time. For this study, cadaver trunk skin was dermatomed to 0.25 mm and mounted on diffusion chambers. The dermal side was bathed by saline, and the epidermal side was left open. [^{14}C]Methyl Alcohol was applied to the epidermal surface, and the receptor solution was analyzed at intervals for radioactive content.

Gummer and Maibach (1986) investigated the in vitro penetration of 25 to 500 μl [^{14}C]Methyl Alcohol and [^{14}C]ethyl alcohol through excised guinea pig skin. Three areas of dorsal skin of three female Hartley guinea pigs was clipped and removed by blunt dissection. Superficial muscle tissue was removed from the skin, but subcutaneous fat was not removed. The skin samples were placed onto flow-through penetration cells (skin surface area = 5 cm^2) with a receptor phase volume of 5 to 5.3 ml. The alcohol was allowed to penetrate over a period of 19 hours, both with occlusion and without. Parafilm laboratory film and Gel Bond polyester film were used for simple occlusion, and a plastic Hill Top chamber was used for complex occlusion. The amount of penetration for each applied volume of Methyl Alcohol is listed in Table 2 as a percent of the applied dose recovered. The total amount of Methyl Alcohol that penetrated doubled between doses of 50 and 100 μl, but not between doses of 25 and 50 μl or 100 and 200 μl. No significant dose-related difference was observed in the total amount of Methyl Alcohol that penetrated when measured as a percentage of the applied dose. With simple occlusion using Parafilm, peak penetration of the alcohol occurred at 3 hours, followed by a steady decrease in the percentage that penetrated per hour. When Gel Bond was used as the occlusive material, Methanol penetration reached a peak (3.0% of applied dose/hour at 6 hours), and then steadily declined. After occlusion with the Hill Top chamber, Methanol recovery in the receptor phase was greatly increased, compared to results for unoccluded or Parafilm-occluded diffusion cells. Additionally, compared to Gel Bond occlusion, use of the Hill Top chamber resulted in a marginal (but statistically significant, $p < 0.05$) increase in the amount of methanol that was recovered in the receptor phase. Peak penetration of methanol (under Hill Top chamber occlusion) was reported as 3.85% of the applied dose per hour at 6 hours. The total recovery of radioactivity was 78%, indicating a significant loss of radioactivity throughout the study.

TABLE 2
Penetration of [^{14}C]Methyl Alcohol through excised guinea pig skin (Gummer and Maibach 1986)

Volume applied (μl)	Cell covering	Penetration (%)
50	none	0.48 ± 0.09
100	none	1.33 ± 0.30
200	none	1.40 ± 0.07
500	none	0.99 ± 0.18
100	Parafilm	13.2 ± 2.7
100	Gel bond	34.8 ± 1.8
100	Hill Top chamber	44.2 ± 3.0

The high water miscibility of Methyl Alcohol allowed the alcohol to be distributed throughout all organs and tissues in direct relation to the body's water compartment (Kavet and Nauss 1990; Von Burg 1994); concentrations of Methyl Alcohol were greatest in the vitreous humor of the globes, kidneys, liver, gastrointestinal tract, and optic nerve, and were smallest in the brain, muscle, and adipose tissue. The apparent volume of distribution was 0.6 to 0.7 l/kg (Bartlett 1950; Ellenhorn and Barcelous 1988). Methyl Alcohol concentrations in the blood continued to increase during exposure, and peaked at or shortly after cessation of exposure, then declined rapidly (Von Burg 1994). On average, the blood Methyl Alcohol concentration was equal to 83% of its aqueous concentration, regardless of the route of exposure. Methyl Alcohol concentrations in urine were 20% to 30% greater than blood (Kavet and Nauss 1990).

In a study by Yant and Schrenk (1937), dogs were exposed to 4000 to 15,000 ppm Methyl Alcohol vapors, 2.5 g/kg Methyl Alcohol via stomach tube (50% in water), or 5 g/kg undiluted Methyl Alcohol via subcutaneous injection into the flank. In the inhalation study, two dogs were exposed to 4000 ppm for 12 hours, one was exposed to 4000 ppm for 5 days, two inhaled 15,000 ppm for 22 to 23 hours, and one inhaled 15,000 ppm for 24 hours. Dogs exposed for 12 and 22 to 23 hours were killed immediately after termination of exposure, dogs exposed for 5 days were killed within an hour of termination of exposure. The dog exposed for 24 hours was allowed to eliminate Methyl Alcohol for 48 hours before being killed, and the dog exposed to the low dose for 5 days was killed 120 hours later. Investigators found "practically the same amount" of Methyl Alcohol in the various organs of the dog exposed to the high dose for 24 hours, as was found in the dogs that inhaled the low dose for 5 days, but considered this finding a coincidence. Variations among the tissues and fluids in the dog that inhaled 15,000 ppm for 24 hours at a particular stage in the elimination process were similar to those in the other test animals, regardless of the exposure conditions. The Methyl Alcohol content of the urine did not differ from the content of the blood, bile, or stomach. When dogs were treated via stomach tube or subcutaneous injection (one per group, killed 5 hours after administration), the relative Methyl Alcohol concentration-water content ratio was similar for all tissues and the blood, and correlated with the results from the inhalation study.

Humans who ingested 71 to 84 mg/kg Methyl Alcohol had blood Methyl Alcohol concentrations of 4.7 to 7.6 mg/100 ml,

and after oral administration of 4 g/kg of Methyl Alcohol, rats had blood Methyl Alcohol concentrations of 100 to 700 mg (Von Burg 1994). During human inhalation studies, exposure to 500 to 1100 ppm for 3 to 4 hours resulted in concentrations of 1 to 3 mg of Methyl Alcohol per 100 ml of urine; the urine/blood concentration ratio was 1.2 to 1.3. Exposure of workers to an average concentration of 200 ppm in air for 8 hours produced an average urinary concentration of 42 mg/l Methyl Alcohol (Von Burg 1994).

Methyl Alcohol is normally found in the body as a result of diet and metabolic processes. Thirty-one unexposed subjects had a mean blood Methyl Alcohol concentration of 0.73 mg/l, and the mean expired Methyl Alcohol from nine "normal" people was 0.25 μg/l. Formate is present in the blood at background concentrations of 3 to 19 mg/l (Kavet and Nauss 1990). In nine unexposed subjects, Eriksen and Kulkarni (1963) reported that the concentration of Methyl Alcohol in the breath was 0.06 to 0.49 μg/l. Methyl Alcohol is also a natural constituent of blood and urine as well as expired air; it is "probably formed" by the activities of intestinal microflora or other enzymatic processes (Šedivec, Mráz, and Flek 1981).

Hepatic metabolism in humans accounts for 90% to 95% of elimination of Methyl Alcohol (Ellenhorn and Barcelous 1988). The following route of metabolism of Methyl Alcohol is believed to be the same for all mammalian species:

$$\text{Methyl Alcohol} \rightarrow \text{Formaldehyde} \rightarrow \text{Formate } (+H^+) \rightarrow CO_2 + H_2O$$

The formation of formaldehyde is catalyzed by hepatic alcohol dehydrogenase and/or a hydrogen peroxide/catalase system. In rats, the latter system predominates, but alcohol dehydrogenase is the key enzyme in humans and nonhuman primates. The initial metabolic step proceeds at similar rates in both primates and rats (Billings and Tephly 1979; Von Burg 1994).

The second reaction occurs as a two-step process. Formaldehyde is oxidized to *S*-formylglutathione in a reaction that requires reduced glutathione (GSH) and is mediated by an NAD-dependent formaldehyde dehydrogenase. In the second step, thiolase catalyzes the conversion of *S*-formylglutathione to formic acid, which dissociates to form formate and a hydrogen ion. The third reaction (formate to CO_2 and water) is catalyzed by catalase (Kavet and Nauss 1990; Von Burg 1994).

Formaldehyde did not accumulate substantially in Methyl Alcohol–intoxicated humans or animals (McMartin et al. 1979; Von Burg 1994), suggesting that the reaction catalyzed by formaldehyde dehydrogenase was not a rate-limiting step (Von Burg 1994). Elevated formaldehyde concentrations in body fluids and tissues have not been detected after administration of Methyl Alcohol (see "The Role of Metabolites in Methyl Alcohol-Induced Toxicity"). Formaldehyde was not detected in blood, urine, or tissues obtained from animals treated with Methyl Alcohol, and humans poisoned with the alcohol had no increased formaldehyde (Kavet and Nauss 1990; Tephly 1991). The time of semitransformation of formaldehyde to formic acid was ~1 minute in many species, including humans, and the metabolite's elimination occurred within 12 minutes in rats and guinea pigs, 67 minutes in dogs, 77 minutes in cats, and 55 minutes in humans (Restani and Galli 1991).

The efficiency of formate metabolism has been linked strongly to the hepatic concentration of tetrahydrofolate (THF) (Von Burg 1994). Formate forms a complex with THF, which is first converted to 10-formyl-THF and then to CO_2. THF is derived from dietary folic acid, and is regenerated in the folate pathway. The folate pathway metabolizes formate in both primates and rats, but the pathway is used more efficiently in rats, such that formate clearance from the blood and its metabolism to CO_2 is approximately two to two and one half times faster in rats than in nonhuman primates (Kavet and Nauss 1990).

Research data have confirmed that 10-formyl-THF dehydrogenase was the catalyst for formate oxidation in the livers of both Sprague-Dawley rats and humans. The investigators concluded that the observed differences in formate metabolism were due to the hepatic concentrations of 10-formyl-THF dehydrogenase (Johlin et al. 1989).

Four female cynomolgus monkeys with both low and normal 5-THF concentrations were exposed to 10 to 900 ppm Methyl Alcohol vapors for 2 hours during disposition studies. The investigators observed that concentrations of Methyl Alcohol–derived formate in blood remained below endogenous concentrations (0.1 to 0.2 μM). In the primates with normal concentrations of 5-THF, the concentrations of Methyl Alcohol–derived [^{14}C]formate in the blood were 10 to 1000 times less than those occurring endogenously (Dorman et al. 1993a; Medinsky and Dorman 1993).

Clay, Murphy, and Watkins (1975) administered 2 and 4 g/kg Methyl Alcohol to five female and one male pigtail monkeys (*macaca nemestrina*); 4 g/kg to four female rhesus monkeys (*macaca mulatta*); and 6 g/kg to Sprague-Dawley rats (number not given). Methyl Alcohol was given intraperitoneally (IP) as a 25% solution in saline. Three rhesus monkeys had no apparent sign of toxicity at 22 to 24 hours, but mild metabolic acidosis was produced; the fourth rhesus monkey developed severe acidosis that led to coma at 18 hours and death at 28 hours. Two pigtail monkeys (treated with 2 to 3 g/kg Methyl Alcohol) had no apparent signs of toxicity, but the remaining four (4 g/kg) became severely acidotic. Two were comatose at 17 to 19 hours and recovered; one was in obvious distress at 15 hours, and was comatose from 18 hours until death at 22 hours. The fourth acidotic pigtail monkey was "weak and apathetic" at 12 hours and comatose at 32 hours (killed at 36 hours). The only observable effect in the rat was transient central nervous system (CNS) depression at 4 to 6 hours. Systemic formate concentrations were low in the rats and rhesus monkeys. Urine collected from rats contained 0.2% of the administered dose as formate, compared to >2.0% in the pigtail monkey (Clay, Murphy, and Watkins 1975). When rats and pigtail monkeys were dosed with <100 mg/kg formate, it cleared with half-lives of 12 and 31 minutes, respectively. The half-times decreased with increasing dosage in both species (Clay, Murphy, and Watkins 1975; Kavet and

Nauss 1990), indicating that formate metabolism was a saturable process (Kavet and Nauss 1990). Pigtail monkeys treated intravenously (IV) with 50 mg/kg 4-methylpyrazole, a hepatic alcohol dehydrogenase inhibitor, prior to administration of 4 g/kg Methyl Alcohol and every 6 hours after Methyl Alcohol dosing appeared normal after 48 hours (Clay, Murphy, and Watkins 1975).

When cynomolgus monkeys were treated with sodium folate or 5-formyl-THF prior to administration of 2 g/kg Methyl Alcohol, the monkeys had a modest increase of blood formate concentration and maintained normal blood pH. Control monkeys given only Methyl Alcohol had a larger increase of blood formate concentration and were temporarily acidotic. In addition, pretreatment with 5-formyl-THF reversed Methyl Alcohol–induced toxicity once it was established (Noker, Eells, and Tephly 1980).

After Methyl Alcohol loading, folate-deficient (FD) rats became acidotic and accumulated folate, whereas monkeys treated with folate supplements (FS) maintained blood pH and formate concentrations. FD rats were unable to oxidize formate, and had signs of Methyl Alcohol toxicity (Maker and Tephly 1977). Lee, Garner, and Terzo (1994) reported that FD rats were more sensitive to Methyl Alcohol exposure than monkeys, and were a congruous animal model for evaluating the effects of Methyl Alcohol in humans.

Black et al. (1985) compared the concentrations of folate intermediates and the activities of folate-dependent enzymes in the livers of Sprague-Dawley rats and cynomolgus (*macaca fascicularis*) monkeys. Total folate content of the two species was similar, but THF concentration in monkey livers was 59% of that of rats.

Human (from cadavers) concentrations of total hepatic folate and THF were 62% and 57% of those of Sprague-Dawley rats, respectively, and 26% and 15% of those of Swiss-Webster mice. The ratio of formate oxidation rate to THF concentration was equivalent for humans, mice, and rats. The enzyme responsible for the final step of the oxidation of formate to CO_2 was reduced in both humans and cynomolgus monkeys (Johlin et al. 1987).

Billings and Tephly (1979) reported that the addition of methionine to Sprague-Dawley rat hepatocyte incubations containing Methyl Alcohol enhanced the oxidation rate of Methyl Alcohol to CO_2 by stimulating the oxidation of formate. Thus, in isolated hepatocytes, the development of a methionine deficiency caused the accumulation of formate during Methyl Alcohol intoxication. The methionine deficiency led to an apparent functional folate deficiency, which reduced the oxidation of formate to CO_2. Hepatocyte cultures obtained from FD rats did not efficiently oxidize formate when 5-THF was added, but oxidation was restored when methionine was added as well.

The half-life of Methyl Alcohol was 1 day or more for high doses (>1 g/kg), and approximately 3 hours for low doses (<0.1 g/kg) (Kavet and Nauss 1990). The excretion of Methyl Alcohol was dose-dependent. Concentrations of Methyl Alcohol in the blood were nondetectable within 4 to 6 and 8 to 10 hours after cessation of exposure using cynomolgus monkeys. The primary route of Methyl Alcohol excretion in the cynomolgus monkey was exhalation (41%–72%), both during and after exposure. Of the remaining absorbed Methyl Alcohol, 2% to 18% was metabolized to carbon dioxide, and 0.1% to 0.76% was excreted in the urine (Von Burg 1994). Eells et al. (1983) treated female cynomolgus monkeys with 1 g/kg [^{14}C]Methyl Alcohol intraperitoneally. Within 24 hours, 78% of the activity was recovered as exhaled CO_2 (Eells et al. 1983). In a study by Kawai et al. (1991), 33 fuel production workers were exposed to Methyl Alcohol vapors. The workers wore gloves to prevent possible skin penetration by the alcohol, but did not wear masks. The mean amount of Methyl Alcohol detected in the urine was 42 mg/l urine after exposure to 200 ppm for 8 hours.

Röe (1955) reported that a "much larger proportion" of Methyl Alcohol than of ethyl alcohol was eliminated in expired air. Dogs fed 5 g/kg expired >50% of the dose and rats fed 1 to 6 g/kg eliminated >70% of the dose during several days after administration of the alcohol. When rats were dosed with 1 g/kg [^{14}C]Methyl Alcohol, 14% was excreted unchanged in the expired air within 48 hours. The rate of oxidation to CO_2 was independent of concentration. Methyl Alcohol was also excreted by the kidneys in amounts as great as 10% of the administered dose.

Methyl Alcohol is eliminated primarily through the lungs (>70% of the dose) and oxidized in the tissues to a smaller extent, whereas the reverse is true for ethyl alcohol (Haggard and Greenberg 1939). The investigators reported that ethyl alcohol was primarily a "reactive substance" and Methyl Alcohol was "nearly nonreactive." As Methyl Alcohol has a high coefficient of distribution between blood and air in the lungs such that its solubility in the blood is great in relation to its vapor tension in the pulmonary air. The amount eliminated in unit time is determined by the concentration of the alcohol in the blood and by the volume of pulmonary ventilation. The rate of elimination of volatile substances from the lungs follows an exponential curve.

In a review of Methyl Alcohol toxicology, Ellenhorn and Barcelous (1988) reported that unchanged renal excretion accounted for 2% to 5% of elimination and that pulmonary excretion accounted for a small amount of elimination when humans ingested the alcohol. Elimination after overdoses followed saturation (zero-order) kinetics.

Cytotoxic Effects

Xie and Harvey (1993) used NG108-15 cell line (rat glioma and mouse neuroblastoma hybrid cell) to test for the neurotoxic effects of Methyl Alcohol and other compounds. The indicator was cell resting membrane potential (RMP). Cell membrane damage caused by toxic compounds altered the structure of the membrane, and thereby disrupted membrane order and function. A decrease in RMP occurred if the ability of cells to maintain ionic gradients was lost. The results of this study were then compared with a parallel 3, [4,5-dimethylthiazd-2-yl]-2,5-diphenyltetrazdium bromide (MTT) assay, which determined

the metabolic activity of viable cells. The RMPs of approximately 30 differentiated cells were measured using an electrometer. At least two cultures were used for each test concentration. The results were expressed as the concentration that caused a 50% reduction from controls (IC_{50}). A concentration-dependent decrease in RMP was observed after 1 or 24 hours of Methyl Alcohol exposure. The IC_{50} of Methyl Alcohol was 1771 mM at 1 hour, and 1281 mM at 24 hours. During the MTT assay, the IC_{50} values were 3731 mM at 1 hour and 826 mM at 24 hours. Previously published IC_{50} values of the MTT assay using other cell types were >1560 mM (Hepatocyte, 3T3, HepG2) and 826 mM (NG108-15). The IC_{50} values from the MTT assays correlated closely with those from the RMP measurements. Methyl Alcohol was more toxic in NG108-15 cells than in other cells (Xie and Harvey 1993).

Koerker, Berlin, and Schneider (1976) added short-chain alcohols, including Methyl Alcohol, and their corresponding aldehydes, including formaldehyde, to cultures of the NBP_2 clone of C1300 mouse neuroblastoma cells to determine the cytotoxicity of the chemicals. The experimental concentrations ranged up to 2 M. Cytotoxicity was determined by making duplicate observations at 2, 12, 24, 48, and 72 hours after exposure: the percentage of cells with "neurite-like" processes, the percentage of cells sloughing in the medium, the total cell number, and the cell viability (as the percentage of cells that excluded 0.1% Trypan blue in phosphate-buffered saline (PBS) after a 2-minute incubation at 37°C). Attached cells with processes longer than ~40 μm were counted using an inverted stage phase-contrast microscope; the processes were similar in appearance to the axons of neurons, and were therefore called neurites.

In this study, the aldehydes were more toxic than the alcohols for each of the effects; formaldehyde was one of the most toxic compounds, regardless of the criteria used to assess cytotoxicity. In general, the most sensitive sign of toxicity was the increased sloughing of cells from the flask surface into the medium. In the case of formaldehyde, the most sensitive indicator of cytotoxicity was a decrease in the percentage of cells having neurites. The concentrations that caused 50% changes from control at 24 hours are listed in Table 3. The length of exposure also affected the observed cytotoxic effects. Low doses that produced little or no toxicity at 24 hours, produced more obvious effects at 48 and 72 hours. These effects, however, were more pronounced for the aldehydes than the alcohols. The investigators concluded that the short-chain aldehydes were more toxic than their corresponding alcohols to neuroblastoma cells in culture, but the concentrations of aldehydes required to produce cytotoxicity were "higher than those which would be expected to occur in vivo."

Other investigators added Methyl Alcohol to cultures of Ascites Sarcoma BP8 and BHK −21/C13 cells to determine the alcohol's cytotoxicity. The mean IC_{50} was 673.0 ± 114.0 mM (Romert, Jansson, and Jenssen 1994) for the Ascites Sarcoma BP8 cells. For the BHK −21/C13 cells, the concentration of Methyl Alcohol that induced a toxic effect during a cell detachment assay was 1230 mM, which corresponded with a low cytotoxicity ranking (Reinhardt, Pelli, and Zbinden 1985). In studies using mouse Balb/c 3T3 fibroblasts, the highest tolerated dose was 864 mM Methyl Alcohol (Borenfreund and Borrero 1984) and the concentration that induced 50% inhibition in [^{3}H]uridine uptake was 668 mM Methyl Alcohol (Shopsis and Sathe 1984). The highest tolerated dose was 1037.4 mM in Chinese Hamster V79 fibroblasts, 617.5 mM in the rabbit cornea epithelium, 111.5 mM in human hepatoma ($HepG_2$) cells, and 1037.4 mM in murine macrophages (RAW 264.7) (Borenfreund and Borrero 1984; Borenfreund and Shopsis 1985).

Miscellaneous Effects

The metabolism of Methyl Alcohol to formate resulted in the inhibition of gluconeogenesis from lactate, thus increasing the tendency to acidosis (Buckley and Vale 1986).

Methyl Alcohol at concentrations greater than 100 mg% (1-hour exposure) caused dose-related isometric contractions of the isolated basilar arteries of 30 dogs. Lower concentrations of Methyl Alcohol had little effect on resting tension. The responses induced by Methyl Alcohol were not mediated by the release of histamine, serotonin, acetylcholine, or norepinephrine. The absence of observed tachyphylaxis suggested a direct vascular action of Methyl Alcohol on canine basilar artery (DeFelice, Delle, and Wilson 1975).

Methyl Alcohol had effects on the heart that were similar to those of ethyl alcohol. Ethyl alcohol caused a negative effect on conductivity, and a decreased threshold for ventricular fibrillation. Oxidation of Methyl Alcohol occurred at a rate independent of blood concentration, but complete elimination required several days. Blood pressure was generally unaffected during human exposures, but the pulse could be slow (Ramos, Chacon, and Acosta 1996).

TABLE 3

Molar concentrations producing 50% change from controls (Koerker, Berlin, and Schneider 1976)

Cytotoxic manifestation	Methyl Alcohol	Formaldehyde
Increased no. of sloughed cells	1.5×10^{-1}	8.3×10^{-6}
Decreased neurite formation	2.0×10^{-1}	2.0×10^{-6}
Decreased viability of sloughed cells	2.8×10^{-1}	4.5×10^{-6}
Decreased total no. of cells	1.3×10^{0}	2.8×10^{-6}
Decreased viability of harvested cells	Not attained	2.2×10^{-4}

The administration of a single IP dose of 3 g/kg Methyl Alcohol to Wistar albino rats (male and female, six per group) resulted in an increase in acetylcholine content of the whole brain from 17.5 ± 2.5 (control) to 37.13 ± 5.33 nM/g at 30 minutes. A corresponding decrease in cholinesterase activity was observed; the control value was 9.942 ± 0.445 nM of acetylcholine hydrolyzed/g/min, and the value in the rats given Methyl Alcohol was 5.9 ± 0.8 nM acetylcholine hydrolyzed/g/min. Two groups of rats (12 total) were given the Methyl Alcohol treatment. Rats of the two control groups were given saline. All the rats were killed 30 minutes after treatment (Babu et al. 1992).

Kobayashi et al. (1995) investigated the immunotoxicity of Methyl Alcohol and other chemicals by examining the cytotoxic function of human natural killer cell-rich populations cultured in vitro. Immunotoxicity was measured as 50% inhibition of cytotoxic activity (IC_{50}) and excretion of rhodamine 123 as measured by flow cytometry. Rhodamine 123 is an indicator of the expression of P-glycoprotein, a multidrug resistance 1 (MDR-1) gene product that is an ATP-driven efflux pump for lipophilic drugs. P-Glycoprotein is expressed at greater concentrations in natural killer cells than other mature lymphoid cells. Blockers of P-glycoprotein inhibit natural killer cell-mediated cytotoxicity. In this assay, Methyl Alcohol had poor inhibitory activity of cytotoxicity, even at a concentration (v/v) of 1%. The IC_{50} of Methyl Alcohol was >500 mM. In addition, Methyl Alcohol did not inhibit rhodamine 123 excretion at concentrations up to 1%.

Methyl Alcohol increased the permeation of ketoprofen across the excised skin of a hairless mouse. Of the compounds tested, Methyl Alcohol had the greatest flux, permeation ratio (percent dose at 24 hours), and enhancing factor. The flux was 244.1 ± 27.1 $\mu g/cm^2/h$, the permeation ratio was 21.9 ± 2.6%, and the enhancing factor was 17.0. The lag time was ~10 hours. In comparison, the hydrophilic vehicles propylene glycol (control) and PBS had flux values of 10.9 ± 2.0 and 24.5 ± 3.0 $\mu g/cm^2/h$, permeation ratios of 1.3 ± 0.2% and 2.7 ± 0.4%, and enhancing factors of 1.0 and 2.1, respectively. The lag times were 7.1 and 6.8 hours, respectively. Binary vehicles containing Methyl Alcohol (with Panasate 800, monoolein, or monocaprylin) also increased significantly the permeability of ketoprofen across excised mouse skin. The maximum enhancement effect occurred when 40% Methyl Alcohol/60% Panasate 800 was evaluated. The lag time was 3.1 hours, the flux was 300.9 ± 25.6 $\mu g/cm^2/h$, the permeation ratio was 44.0 ± 4.9%, and the enhancing factor was 34.1 (Goto et al. 1993).

Undiluted Methyl Alcohol inhibited the metabolic cooperation of cultured Chinese hamster V79 cells when volumes of 25 to 200 μl/5 ml were tested. Gap-junctional intercellular communication is necessary for the regulation of cell proliferation, differentiation, and gene expression (Chen et al. 1984).

Formic acid in the kidneys reportedly interfered with the tubular reabsorption mechanism for calcium. In addition, renal ammoniagenesis in the proximal tubule cells was markedly increased by chronic metabolic acidosis (Liesivuori and Savolainen 1991).

Rat paw edema induced by the injection of carrageenan (0.1 ml of 1% preparation) was inhibited by oral doses of Methyl Alcohol and ethyl, propyl, and butyl alcohols (Strubelt and Zetler 1980). This study used six male Wistar rats per group, and the alcohols were administered by gavage as 10%, 20%, or 40% solutions in distilled water (15 ml/kg; 1.2 to 4.8 g/kg). A dose of 4.8 g/kg Methyl Alcohol caused 26% inhibition of edema, and 1.2 to 2.4 g/kg Methyl Alcohol both caused 7% inhibition, as compared to the untreated control group. In addition, the alcohols had an anti-inflammatory effect upon carrageenan-induced pleurisy by reducing the volume of pleural exudate and the total number of leukocytes in the exudate. In this study, Methyl Alcohol only caused a slight reduction of the cell count.

ANIMAL TOXICOLOGY

In general, only nonhuman primate species present a model of acute human Methyl Alcohol toxicity (Kavet and Nauss 1990). The lethal dose for nonprimates was 2 to 3 times greater than the 3-g/kg lethal dose reported for rhesus monkeys, and was 6 to 10 times greater than lethal doses in humans (see "Clinical Assessment of Safety"). Of all species tested, only nonhuman primates had a sequence of early inebriation and a day latency, followed by acidosis, ocular toxicity, and other signs characteristic of human exposure. After the toxic syndrome was manifested, the primates died. Other species (Sprague-Dawley rats, mice, rabbits, dogs) had an initial narcosis from which they either survived or died; acidosis was not a prominent sign of toxicity (Gilger, Potts, and Johnson 1952; Gilger and Potts 1955; Tephly and McMartin 1984). Dorman et al. (1993b) reported that female minipigs (minipig YU) given Methyl Alcohol did not develop optic nerve lesions, toxicologically significant formate accumulation, or metabolic acidosis. Visual impairment was not observed in nonprimate species poisoned with Methyl Alcohol (Gilger and Potts 1955; Tephly and McMartin 1984). No evidence of visual disturbances or changes in the appearance of the fundus were observed during studies using rabbits, chickens, dogs, and rats (Tephly and McMartin 1984).

Acute Toxicity

Oral

Von Burg (1994) reported an acute oral LD_{50} of Methyl Alcohol in the rat of 5.6 g/kg and in the mouse of 7.3 g/kg. In a study using five albino Swiss mice per group, the oral LD_{50} in males of Methyl Alcohol ranged from 9.5 to 15.3 g/kg (approximate lethal dose = 11.3 g/kg). The no-effect level (NOEL) was 7.5 g/kg. The IP LD_{50} in females ranged from 4.2 to 6.3 g/kg (approximate lethal dose = 5.0 g/kg), and the NOEL was 4.1 g/kg (Paumgartten et al. 1989).

Dermal

The acute dermal LD_{50} of Methyl Alcohol using rabbits was 15.8 g/kg (NAS 1995).

McCord (1931) applied gauze pads treated with Methyl Alcohol to clipped skin sites of eight rhesus monkeys. A dose of 15 ml ("diluted in proportions recommended for antifreeze use") applied four times daily resulted in "profound sickness" on the first day, with an inability to stand and vomiting, and death on the second day. Similar signs of toxicity and death occurred within two days when doses of 0.5 and 1.3 ml were administered four times daily. Methyl Alcohol was recovered from the urine and tissues of all the test animals; formaldehyde was not recovered. The histopathologic examination was performed by Scott, Helz, and McCord (1933). The characteristic lesions caused by Methyl Alcohol were degeneration of parenchymal tissues and neurons, which was accompanied by generalized capillary engorgement and edema. Degeneration of the ganglion cells of the retina was sometimes followed by degenerative changes and fibrosis of the optic nerve.

Inhalation

The acute inhalation LC_{50} values of Methyl Alcohol using rats ranged from 64 to >145 g/kg after 1 to 4 hours of exposure (Von Burg 1994; NAS 1995). The LC_{50} of Methyl Alcohol using cats was 33.6 g/kg (6 hours), and using mice, the LC_{50} was 61.1 g/kg (134 min) (Von Burg 1994).

McCord (1931) exposed 11 rhesus monkeys to Methyl Alcohol vapors at concentrations of 1000 to 40,000 ppm/day for up to 18 hours per day for 6 days. Three monkeys were exposed to 40,000 ppm, one was exposed to 20,000 ppm, two were exposed to 10,000 ppm, one inhaled 5000 ppm, and four were exposed to 1000 ppm. "Obvious sickness" was noted within 2 to 3 days after exposure to the high dose (1–4 h/day), and death occurred soon afterward. Monkeys exposed to 10,000 ppm for 7 h/day survived, but those exposed to the same concentration for 18 h/day died. The toxic concentration was <1000 ppm, but marked differences in individual susceptibilities were observed. Methyl Alcohol was regularly recovered from the urine and tissues of the experimental animals, whereas formaldehyde was not recovered. The histopathologic study was performed by Scott, Helz, and McCord (1933). The observed lesions and signs of toxicity were degeneration of the parenchymal tissues and neurons, generalized capillary engorgement and edema, degeneration of the ganglion cells of the retina, and degenerative changes and fibrosis within the optic nerve.

Ocular

The ocular toxicity of Methyl Alcohol after administration to nonhuman primates by various routes has been well documented (Tephly and McMartin 1984; Von Burg 1994). Common to Methyl Alcohol poisoning were findings of dilated and unresponsive pupils, optic disc hyperemia and edema, retinal edema, and retinal ganglion cell degeneration. When rhesus monkeys were treated with Methyl Alcohol or its metabolites, ocular toxicity was evident after 40 hours of exposure (Hayreh et al. 1977; Tephly and McMartin 1984).

Four male rhesus monkeys were given an IV formate infusion using a loading dose of 1.25 mmol/kg sodium formate (Martin-Amat et al. 1978) to determine the effects of formate. Two monkeys served as controls. Formate buffers (sodium formate:formic acid, 10:1, 0.5 M) were infused by indwelling catheter of the femoral vein after the loading dose. The rate of infusion, 3.1 mEq/kg/h, was calculated to produce concentrations of formate similar to those observed in Methyl Alcohol–intoxicated monkeys that had ocular lesions. Blood samples (from the femoral artery) were analyzed for arterial blood formate, formaldehyde, pH, and pCO_2. Cerebrospinal fluid samples were analyzed for formate and formaldehyde. Pupillary reflexes were tested periodically throughout the study, and fundus photography was performed. Microscopic examination of the retina, optic disc, and optic nerve was made.

After 10 hours, the monkeys of the treatment group had blood formate concentrations of 10 to 30 mEq/l. In general, a correlation between rate of onset of ocular toxicity, degree of toxicity, and the blood formate concentration was observed (Table 4). Blood pH values were 7.4 to 7.6 in all treated monkeys. Pupillary reflexes were rapidly altered in monkeys given formate, and most animals had no response to light by 24 and 48 hours after infusion began. Marked optic disc edema was observed, and was localized mainly in the prelaminar region. The optic nerve lesions were mostly in the central portion of the proximal part of the nerve, and generally did not continue to the distal part of the optic nerve. The retina, including the ganglion cell layer, was not affected by treatment with formate.

FD rats given Methyl Alcohol had lesions of retinal toxicity, induced by the depletion of ATP (Garner, Lee, and Louis-Ferdinand 1995a). The Müller cell, which is the primary retinal glial cell necessary for the support of retinal neurons, was

TABLE 4
Ocular toxicity of Formate (Martin-Amat et al. 1978)

Monkey	Time after initiation of infusion (h)	Maximum blood formate concentration (mEq/l)	Pupillary reflex	Fundus changes
1	39	34	No response, mydriasis 7 mm	Moderate optic disc edema with retinal edema
2	50	30	No response, mydriasis 8 mm	Severe optic disc edema
3	41	20	No response, mydriasis 8 mm	Severe optic disc edema
4	25	12	Normal, 3 mm	Moderate optic disc edema

likely the initial target in Methyl Alcohol–induced ocular toxicity. This cell contains the enzymes needed to metabolize Methyl Alcohol. The Müller cell can behave as a K^+ electrode by shunting high concentrations of K^+ from the extracellular space through its endfoot region, which has the greatest degree of membrane conductance of the cell. In humans, nonhuman primates, and FD rats, a single dose of Methyl Alcohol caused a severe decrease in b-wave amplitude at 48 hours. The b-wave amplitude was visualized using the electroretinogram (ERG), and was produced by the Müller cell in response to an increase in extracellular potassium released by the bipolar cell. These investigators also used in vivo Müller cell function to evaluate the effect of Methyl Alcohol on metabolic and electrophysiological indices of ocular toxicity.

Adult, male Long-Evans hooded rats were given 1.0 to 3.0 g/kg Methyl Alcohol by gavage in a 30% aqueous solution. The rats were maintained on either a FD or FS diet for 13 weeks prior to testing. The positive controls were the Müller cell toxin α-aminoadipic acid (five rats), potassium cyanide (number not given), 2-amino-4-phosphonobutyric acid (five rats), sodium iodate (three rats), and the potassium channel blockers tetraethylammonium and cesium (three rats each). These controls were injected intravitreally to rats not treated with Methyl Alcohol. The untreated eye was injected with vehicle, and served as the negative control. Following treatment with Methyl Alcohol, retinas were collected for determination of the retinal ATP concentration. ERGs were performed on anesthetized rats after the sensory hairs were trimmed and the pupils were dilated with 1% tropicamide. Next, the potassium-induced Müller cell depolarization (KIMD) was measured. A 30-gauge needle was inserted through the cornea and was positioned on the retinal side of the inner limiting membrane. A pulse of potassium chloride was injected into the retina, and the resultant signal was recorded using the ERG electrode.

FD rats given 3.0 g/kg Methyl Alcohol had a 21% decrease in retinal ATP compared with rats given water alone. No difference in retinal ATP was observed in any rats given water alone (six rats) or in FS rats given Methyl Alcohol. The effects of α-aminoadipic acid and cyanide did not differ when rats were maintained on an FD diet as compared to those given FS feed. No KIMDs were observed in any of the rats given α-aminoadipic acid. The eyes of FD rats given cyanide had a 47% decrease in retinal ATP after a 1-hour pretreatment compared to the untreated eyes. Pretreatment with sodium iodate (a pigment epithelium toxin) had no effect on KIMD. Treatment with the K^+ channel blockers blocked the generation of the response to exogenously applied K^+. No KIMDs were observed in FD rats treated with Methyl Alcohol, and an a-wave–only ERG was observed at 48 hours post dose. No change in the elicitation of KIMDs was observed in any rat given water, or in FS rats given Methyl Alcohol. For FD rats, the NOEL was 1.0 g/kg, and the KIMD response was completely eliminated at 2.5 g/kg. The investigators concluded that the Methyl Alcohol-induced decrease in retinal ATP closely paralleled the 20% decrease observed in α-aminoadipic acid–treated retinas. The loss of b-wave generation suggested that the Müller cell was the target of retinotoxicity induced by Methyl Alcohol.

In a related study, also using FD and FS Long-Evans hooded rats, Garner et al. (1995b) reported that intraretinal metabolism of Methyl Alcohol was necessary for the formate-mediated initiation of Methyl Alcohol–induced retinal toxicity. The rats (five per group) were given 2.5 to 3.0 g/kg of Methyl Alcohol at a concentration of 30% by gavage. Disulfiram (as a suspension in 0.5% methyl cellulose and saline) was administered 4 hours prior to Methyl Alcohol treatment, at a dose of 75 mg/kg IP. At this dose, disulfiram was an inhibitor of aldehyde dehydrogenase activity by ~75% at 4 hours post treatment, and by 50% at 40 hours. The rats were also infused via the jugular vein with pH-buffered formate at a rate of 0.5 mmol/kg/h for 48 hours. ERG testing was performed, and the retinas and vitreous humor were collected for Methyl Alcohol and metabolite analyses.

Blood (from the tail vein) Methyl Alcohol concentrations were not significantly different in FD rats as compared with FS control rats. FS rats given Methyl Alcohol had a small increase in blood formate at all time points measured (Table 5). Formaldehyde was not detected in the blood of FD and FS rats given 3.0 g/kg Methyl Alcohol, and was also not detected in the blood of FD rats given disulfiram. Methyl Alcohol concentrations in the retina and vitreous humor were similar in rats of all treatment groups. The FD rats given the high dose of Methyl Alcohol had increased blood and vitreous humor formate concentrations compared to FS rats given the same dose of Methyl Alcohol. Disulfiram treatment decreased the formate concentration in the vitreous humor and retina of FD rats given 3.0 g/kg Methyl Alcohol. FS rats and FD rats given disulfiram had normal ERGs at 48 hours after Methyl Alcohol administration (3.0 g/kg), as did rats given disulfiram alone. Methyl Alcohol–treated FD rats that did not receive disulfiram had a-wave–only ERGs. FD rats infused with formate had blood formate concentrations similar to those observed with retinotoxic doses of Methyl Alcohol, but the ERG responses did not differ from those of FD control rats given water instead of Methyl Alcohol.

Murray et al. (1991) reported that male Long-Evans rats (six per group) injected IP with 4.0 g/kg Methyl Alcohol had formic acidemia and signs of ocular toxicity. In the ERGs, a significant early deficit was observed in b-wave amplitude, followed by a delayed, lesser reduction in a-wave amplitude. At microscopic examination of the globes 60 hours after administration of Methyl Alcohol, generalized retinal edema and vacuolation of the photoreceptor cells and retinal pigment epithelium were observed. Swelling and disruption of the mitochondria in photoreceptor inner segments, optic nerve, and the retinal pigment epithelium were observed in electron micrographs.

In a separate study, male Long-Evans rats treated with nitrous oxide gas prior to administration of 20% Methyl Alcohol in saline (4 g/kg IP initially, 2 g/kg supplements injected at 18 and 42 hours) had formate accumulation and metabolic acidosis, and had disruption of visual and retinal function. Nitrous oxide depleted hepatic THF by inactivating the enzyme methionine synthase, which is needed for the production of THF.

TABLE 5
Methyl Alcohol and Formate tissue concentrations in rats at 48 hours as a function of Folate and Methyl Alcohol in diet (Garner et al. 1995b)

Treatment	Chemical	Mean tissue concentration		
		Blood (mM)	Vitreous humor (mM)	Retina (μmol/g)
Folate deficient diet + water	Methyl Alcohol	0.4 ± 0.01	0.10 ± 0.05	0.09 ± 0.04
	Formate	0.10 ± 0.02	0.12 ± 0.03	0.05 ± 0.01
Folate supplemented diet + 3.0 g/kg Methyl Alcohol	Methyl Alcohol	32.22 ± 2.40	13.85 ± 1.40	5.87 ± 0.15
	Formate	0.59 ± 0.49	1.51 ± 0.14	1.21 ± 0.06
Folate deficient diet + disulfiram + 3.0 g/kg Methyl Alcohol	Methyl Alcohol	39.70 ± 3.21	14.33 ± 0.53	6.85 ± 0.56
	Formate	3.20 ± 0.50[a]	4.70 ± 0.26[a]	3.04 ± 0.19[a]
Folate deficient diet + 3.0 g/kg Methyl Alcohol	Methyl Alcohol	35.13 ± 2.95	18.52 ± 1.47	6.06 ± 0.64
	Formate	14.55 ± 0.73[b]	19.52 ± 0.85[b]	15.28 ± 1.61[b]
Folate deficient diet + formate infusion	Formate	14.18 ± 0.71	10.76 ± 0.68[b]	2.50 ± 0.26[b]

[a]Different from above treatment group ($p < .05$).
[b]Different from above treatment group ($p < .001$).

Visual effects were determined by measuring reductions in the flash-evoked cortical potential (FEP) and changes in the ERG. At 60 hours after Methyl Alcohol administration, the FEP amplitude was approximately 60% that of the control (saline). The ERG was undetectable in rats given Methyl Alcohol at 42 hours after treatment. The observed reductions in FEP and ERG amplitudes occurred coincident with the accumulation of blood formate (Eells 1991).

When female minipigs (3 per group, total 12) received Methyl Alcohol by gavage at concentrations of 1.0 to 5.0 g/kg, the minipigs had dose-dependent signs of acute Methyl Alcohol intoxication. The signs included mild CNS depression, tremors, ataxia, and recumbency that developed within 0.5 to 2 hours, and resolved by 52 hours. Acidosis, formate accumulation, and optic nerve lesions were not observed. The investigators concluded that the minipig was not overtly sensitive to Methyl Alcohol, and was not a suitable animal model for acute Methyl Alcohol–induced neuroocular toxicosis (Dorman et al. 1993b).

Intravenous

In cats, the IV lethal dose of 5% Methyl Alcohol was 118 ml/kg, which corresponded to 5.9 ml/kg of the pure alcohol (Macht 1920). In contrast, 5% concentrations of ethyl, propyl, and butyl alcohols had lethal doses of 100 ml/kg (5 ml/kg pure), 40 ml/kg (2 ml/kg pure), and 6 ml/kg (0.3 ml/kg pure), respectively. Amyl, isopropyl, isobutyl, isoamyl, and benzyl alcohols were also more toxic than Methyl Alcohol. The investigator concluded that the toxicities of a homologous series of alcohols increased in proportion to their molecular weights (Richardson's rule). In subsequent investigations (Röe 1955), it was found that this was true only for certain experimental animals, as a dose of 1 g/kg of Methyl Alcohol can produce blindness and death in humans.

Short-Term Inhalation Toxicity

Four male Sprague-Dawley rats per group were exposed to 0.2 to 10 g/kg Methyl Alcohol vapors for up to 6 weeks (6 h/day, 5 days/week), and then were killed for biochemical and cytological evaluations. Additional groups of rats were killed 2 weeks later. The lungs were collected and examined for biochemical and cellular effects. No significant changes of the lung surface or parenchyma were detected after exposure to up to 10 g/kg of Methyl Alcohol (White et al. 1983).

Cynomolgus monkeys were exposed to Methyl Alcohol vapors for 6 h/day, 5 days/week over a period of 4 weeks. The exposure concentrations were 650, 2600, and 6500 mg/m^3. The monkeys were observed for signs of toxicity twice daily, and were given complete physical examinations each week. Ophthalmoscopic examinations were performed prior to the testing period and at termination. The monkeys were killed, examined, and weighed. Tissues (nasal turbinates, trachea, lungs, esophagus, liver, and globes and optic nerves) from monkeys of the control and high exposure groups were examined microscopically. No treatment-related effects were observed in cynomolgus monkeys of any group (Andrews et al. 1987).

Dermal Effects

In electron micrographs, marked alterations of the nuclear and organelle membranes occurred after topical treatment with a 2:1 chloroform-Methyl Alcohol solvent. The solvent damaged the stratum corneum cells of the epithelium. Treatment with Methyl Alcohol alone, however, produced a slight increase of permeability by removing cutaneous lipids; the alcohol did not alter barrier structure (Vinson et al. 1965).

Ocular Irritation

Carpenter and Smyth (1946) reported that 0.5 ml of undiluted Methyl Alcohol caused severe injury to the corneas of five

albino rabbits after application to the center of the cornea. The observed injury included necrosis (visible after staining) covering more than three fourths of the surface cells of the cornea, or deeper necrosis covering a smaller area. Treatment with 0.1 ml of Methyl Alcohol caused less extensive necrosis that involved as much as three fourths of the corneal surface.

Undiluted Methyl Alcohol was classified as a moderate ocular irritant when corneal pachymetry was performed 3 days after instillation of the alcohol into the eyes of seven Stauffland Albino rabbits (New Zealand white and Florida white cross). A 0.1-ml sample of the alcohol was instilled into the lower conjunctival sac of the left eye of each rabbit. The rabbits were observed prior to treatment and on days 1, 2, 3, 4, and 7, then every 3 to 4 days until 21 days after application of Methyl Alcohol. Conjunctival irritation was scored by direct observation, and iris and corneal irritation were evaluated using a slit-lamp biomicroscope. A pachymeter attachment was used to measure the thickness of the treated and untreated corneas. Conjunctival irritation was scored using the Draize scoring method and corneal cloudiness and inflammation of the iris were scored using the MacDonald scoring criterion. In contrast, when scored using EPA criteria based on the Draize test, Methyl Alcohol was classified as corrosive (Morgan, Sorenson, and Castles 1987).

The ocular irritancy of Methyl Alcohol was evaluated by Muir (1983) using isolated ileum from five California white rabbits. The in vitro assay determined the concentration needed to block spontaneous contractions of the ileum by 50% (EC_{50}), and was compared with the in vivo assay performed by Carpenter and Smyth (1946). The EC_{50} of Methyl Alcohol was 1.38% ± 0.45 (v/v), which corresponded well with the in vivo results.

Nagami and Maki (1993) compared the results of the Draize ocular irritation assay with those of the MTT cytotoxicity assay using normal human epidermal keratinocytes. In this study, the maximum concentration of Methyl Alcohol that did not cause ocular irritation in three to six rabbits was 29% (w/v). The IC_{50} in the in vitro MTT assay was 92,800 μg/ml. The correlation coefficient between the two assays was 0.99.

The scores of an in vitro cytotoxicity assay (inhibition of uridine uptake) using mouse Balb/c 3T3 cells (see "General Biology—Cytotoxic Effects") corresponded to in vivo assays (Draize test) in which Methyl Alcohol was a mild to moderate ocular irritant (Borenfreund and Shopsis 1985).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Rats

Nelson et al. (1984) reported that inhalation of 20,000 ppm Methyl Alcohol caused an irregular gait in exposed dams, and a decrease in mean body weight. The dams, 15 Sprague-Dawley rats per group, were exposed for 7 h/day until gestation day (GD) 19. An increased incidence of urinary and cardiovascular defects, and extra or rudimentary cervical ribs were observed in offspring of this group. Similar malformations were observed in offspring of dams that inhaled 10,000 ppm Methyl Alcohol, but the incidence was not significantly different from that of the control group. The NOEL was 5000 ppm Methyl Alcohol.

Pregnant Sprague-Dawley rats were exposed to 5000, 10,000, or 20,000 ppm Methyl Alcohol vapor (99.1% pure) on GD 1 to 19 during a teratologic assessment of the alcohol (Nelson et al. 1985). The rats were treated for 7 h/day, and were kept in the chambers for approximately 30 minutes after the termination of vapor generation before being returned to their cages. Feed and water were available ad libitum. Blood concentrations of Methyl Alcohol were determined after concurrent exposure on days 1, 10, and 19 using nonpregnant rats. These rats were killed, and approximately 5 ml of blood was removed from the inferior vena cava for analysis. The pregnant rats were weighed and killed on GD 20. The uterus (with attached ovaries) was removed, and the numbers of corpora lutea, resorptions, and live fetuses were determined (Table 6). The fetuses were serially removed and examined for external malformations, sexed, and weighed; the fetuses were then divided into two groups. Fetuses of the groups were examined for skeletal or visceral malformations, respectively.

Even at the greatest test concentration, Methyl Alcohol was not severely toxic to the dams. Pregnant rats exposed to 20,000 ppm Methyl Alcohol had slightly unsteady gait after the initial days of exposure, but feed and water consumption and body weights were not significantly affected. No adverse effects were observed in dams that inhaled 5000 or 10,000 ppm Methyl Alcohol. The blood concentration of Methyl Alcohol of rats exposed to 20,000 ppm was just under 9 mg/ml. The blood concentration of rats dosed with 10,000 ppm Methyl Alcohol was approximately 2 mg/ml.

Treatment with Methyl Alcohol did not affect the numbers of corpora lutea or implantations, or the percentage of dead or resorbed fetuses. The two largest concentrations caused dose-related decreases in fetal weight. Increases in the incidence of malformations after exposure to Methyl Alcohol also were observed. Nine fetuses from four litters of the 20,000 ppm group had external malformations (three exencephaly and six encephalocele). In addition, 14 of 15 litters of this group had one or more animals with skeletal malformations, and 10 of 15 litters had offspring with visceral malformations. Overall, a dose-dependent decrease of the frequency of normal fetuses was observed as the Methyl Alcohol exposure concentration was increased. The incidence of visceral variants did not vary among groups, but more skeletal variants were observed in offspring of the 10,000 ppm (22% of fetuses affected) and 20,000 ppm (69%) groups compared to controls (12%). These differences were not significant, however, when the values were corrected for multiple comparisons. The researchers concluded that Methyl Alcohol was teratogenic at high concentrations when administered to rats by inhalation. The NOEL was 5000 ppm Methyl Alcohol.

Nelson, Brightwell, and Krieg (1989) reported that both male and female Sprague-Dawley rats exposed to >5000 ppm Methyl Alcohol vapor produced offspring with unspecified malformations.

TABLE 6
Developmental toxicity via inhalation—rats (Nelson et al. 1985)

	Control	5000 ppm	10,000 ppm	20,000 ppm
No. of pregnant rats	15	13	15	15
Mean no. of corpora lutea/dam	15 ± 1	16 ± 2	17 ± 1	not collected
Mean no. of implants/dam	15 ± 1	16 ± 2	16 ± 1	14 ± 1
% implants resorbed	8	4	5	10
F/M sex ratio	53:47	56:44	52:48	58:42
Mean female fetal weight (g)	3.15 ± 0.32	3.19 ± 0.24	2.93 ± 0.26[a]	2.76 ± 0.47[a]
Mean male fetal weight (g)	3.34 ± 0.36	3.30 ± 0.24	3.12 ± 0.30[a]	2.82 ± 0.56[a]
No. of fetuses (litters) in skeletal exam group	98 (15)	90 (13)	115 (15)	92 (15)
No. of fetuses (litters) with skeletal malformations	0	1 (1)	2 (2)	72 (14)
No. of fetuses (litters) in visceral exam group	107 (15)	90 (13)	107 (15)	96 (15)
No. of fetuses (litters) with visceral malformations	0	2 (1)	2 (2)	15 (7)
Total no. of fetuses (litters) examined	205 (15)	180 (13)	222 (15)	188 (15)
No. of litters (% fetuses) with skeletal malformations	0	1 (1)	2 (2)	14[a] (79)
No. of litters (% fetuses) with visceral malformations	0	1 (3)	5 (8)	10[a] (29)
No. of litters (% fetuses) with skeletal variations	8 (12)	12 (27)	11 (24)	14 (69)
No. of litters (% fetuses) with visceral variations	11 (21)	7 (11)	13 (26)	12 (26)
No. of litters with abnormal[b] fetuses	0	2	7	14
% litters with abnormal[b] fetuses	0	15	47	93[a]
% normal fetuses	100	98	96	46[a]

[a]Significantly different from the control group, $p < .05$.
[b]Abnormal = having skeletal or visceral malformations.

In an investigation of the toxicokinetics of Methyl Alcohol, Ward and Pollack (1995) reported that fetuses of pregnant Sprague-Dawley rats injected with Methyl Alcohol (IV bolus, 100 or 500 mg/kg; IV infusion, 100 or 1000 mg/kg) had approximately 25% greater concentrations of the alcohol at distribution equilibrium than the dams. The initial rate of fetal permeation decreased linearly with increasing maternal blood Methyl Alcohol concentration. The data indicated that the alcohol could decrease blood flow to the fetal compartment in a concentration-dependent manner, which would decrease the rate of Methyl Alcohol presentation to the conceptus. In this study, fetal Methyl Alcohol concentrations were determined using intrauterine microdialysis of the amniotic fluid and gas chromatography.

Three groups of pregnant rats were treated with 1.3, 2.6, and 5.2 ml/kg Methyl Alcohol orally on GD 10 to 20. One group of pregnant rats served as an untreated control group. No evidence of maternal toxicity was observed. A significantly greater proportion of affected litters occurred at 1.3 and 2.6 ml/kg. The most consistent anomaly observed in the offspring of treated rats was hemorrhage, but the incidence was not reported (Youssef 1991).

Mice

Methyl Alcohol inhalation caused phase-specific developmental toxicity after pregnant Crl:CD-1 ICR BR (CD-1) mice were exposed during organogenesis (GD 6–15), the period of neural tube development and closure (GD 7–9), or the time of potential neural tube reopening (GD 9–11) (Bolon 1993; Bolon et al. 1993; Bolon, Welsch, and Morgan 1994).

In one study (Bolon et al. 1993), 5 to 12 dams per group were exposed for 6 h/day to Methyl Alcohol vapors at concentrations of 5000, 10,000, or 15,000 ppm. On GD 17, the mice were killed. The uterus was removed from each, and the numbers of live and dead fetuses, implantation sites, and resorptions were counted. The fetuses were removed and examined for skeletal and visceral malformations. In addition, pregnant mice were exposed to 0 or 15,000 ppm Methyl Alcohol on GD 7 to 9 to determine if the observed neural tube defects were the result of aberrant closure. These mice were killed on GD 9 or 9.5, and the embryos were examined for neural tube defects using a dissecting microscope.

On the first, second, and third days of exposure, approximately 20%, 10%, and 5% of dams had neurologic signs (ataxia, circling, tilted heads, depressed motor activity), respectively. Three mice were removed from the study on GD 7 due to the severity of the clinical signs, but these mice had no visible lesions. The remaining affected dams recovered within 12 hours after exposure ended. No signs of toxicity were observed in mice of the 5000 or 10,000 ppm groups. Maternal body weights on GD 17 decreased after exposure to 10,000 ppm during GD 6 to 15, or after exposure to 15,000 ppm Methyl Alcohol on GD 7, 7 to 8, or 7 to 9. The observed weight losses were partly due to high resorption rates, which were increased in litters of dams

that inhaled 10,000 ppm (GD 6–15 or 7–9) or 15,000 ppm (GD 7, 7–8, or 7–9).

The body weights of near-term fetuses (GD 17) were reduced in litters of dams exposed to either 10,000 ppm Methyl Alcohol on GD 6 to 15, or 15,000 ppm on GD 7 to 8 or 7 to 9. Methyl Alcohol-treated dams had a higher percentage of litters with malformed fetuses for all exposure periods. The most common finding was renal pelvic dilatation, and was observed in mice of both control and treated groups. Fetuses of dams given 10,000 to 15,000 ppm had neural tube defects, cleft palate, kinked tails, and digit anomalies. The incidence of malformations was dose-dependent. Dams that inhaled the greatest dose had malformed fetuses, regardless of maternal toxicity.

The observed teratogenic effects varied with the timing and duration of exposure. Neural tube defects occurred when dams were exposed during GD 7 to 9; limb abnormalities occurred during GD 9 to 11. Cleft palate and hydronephrosis were observed after dams were treated during either developmental period. Neural tube defects were most likely to develop after a single 6-hour exposure to 15,000 ppm Methyl Alcohol on GD 8. Inhalation on GD 7 resulted in approximately threefold more fetal resorptions, but the incidence of neural tube defects (relative to the GD 8 group) was halved. A single exposure on GD 9 did not result in neural defects, and few resorptions were observed.

The incidence of neural tube defects was 12% to 25% per litter. The most common lesion (found in 91% of affected fetuses) was exencephaly. Other defects included anencephaly, holoprosencephaly, and encephalocele (3% of affected fetuses each). The cerebral cortices, midbrain, and cerebellum were absent in fetuses with anencephaly.

Exposure to Methyl Alcohol also caused grossly visible ocular anomalies. The most common defect (5.4% of fetuses) was premature opening of the eyelids with exophthalmos of one or both globes. This group included 44% of fetuses with exencephaly and 3.5% of fetuses with grossly normal brains. Maternal inhalation on GD 7 to 9 also caused malformations of the palate (14%), tail (14%), skull (7.3%), and jaw (0.1%). Offspring of dams that inhaled 15,000 ppm Methyl Alcohol had both tail malformations and limb defects; digit anomalies were four times more frequent than defects of the paw, and affected multiple fetuses per litter.

In a second study using mice, Bolon (1993) reported that Methyl Alcohol–induced neural tube defects were likely due to the aberrant closure of the anterior neuropore. Methyl Alcohol and formate were the toxic agents that contributed to the neurotoxicity by primarily targeting mesenchymal cells. The period of neurulation, GD 7 to 9, was the period of greatest vulnerability.

Similar findings were reported in a third study using CD-1 mice (Bolon, Welsch, and Morgan 1994). The results suggested that exposure of dams to high concentrations of Methyl Alcohol (15,000 ppm) injured multiple stem cell populations in the neurulating mouse embryo, and that significant neural lesions can remain in older conceptuses, even in the absence of gross lesions. In this study, embryos of dams exposed for 6 h/day on GD 7 to 9 had stunting, delayed rotation, and microcephaly (>90%), and persistent patency of the anterior neurophore and prosencephalic hypoplasia (>40%–90%). Shallow optic vesicles, stunted branchial arches, scoliosis, and hydropericardium were observed. Many 10.5-day-old embryos had edema of the cranial mesenchyme, and at least 21% of the GD 9.5 and 10.5 embryos had occult dysraphism associated with narrowing and angularity of the mesencephalon.

Dorman et al. (1995) determined that Methyl Alcohol–induced exencephaly in offspring of treated female CD-1 mice was the direct result of the effects of the parent compound (administered via inhalation at 10,000 to 15,000 ppm or by gavage at 1.5 g/kg) rather than the accumulation of formate.

Pregnant CD-1 mice were exposed to 5000 and 15,000 ppm (study 1) or 2000 and 5000 ppm (study 2) Methyl Alcohol vapors for 7 h/day on GD 6 to 15. The mice had water ad libitum, but not feed during exposure. Sham-exposed, unexposed, and feed-deprived controls were included. No signs of maternal toxicity were observed, but mice of all exposure groups gained less weight than fed or feed-deprived unexposed control mice. Most of the litters of dams exposed to 15,000 ppm were totally resorbed, and 38% of fetuses that survived to day 17 had exencephaly. Exposure to 5000 ppm in both studies resulted in exencephaly; approximately one third of the litters and 5%–10% of the fetuses were affected. Of the mice given 2000 ppm Methyl Alcohol, 1 of 220 fetuses had exencephaly. No exencephaly occurred in mice of the control groups. The maternal plasma concentrations of Methyl Alcohol were 2000 and 8000 μg/ml at the end of the first period of exposure to 5000 and 15,000 ppm, respectively (Rogers, Chernoff, and Mole 1991).

The administration of 4.0 to 5.0 g/kg (total) Methyl Alcohol to pregnant mice before GD 9 caused dose-dependent skeletal malformations in the fetuses (Connelly and Rogers 1994). The mice were treated by gavage in two doses on GD 7. The dams were killed on GD 18 and the fetuses were counted, weighed, and examined. Vertebral and rib characteristics were observed, and the cervical vertebrae were disarticulated for other analyses. None of the mice of the control group had malformations. Fetuses of mice given 5.0% Methyl Alcohol had full ribs on C7 (i.e., attached to sternum; 30%), C5 with tubercula anterior (normally found on C6; 26%), and split and/or incomplete C2 (left, 30% right, 15%). Fetuses of mice given 4.0% Methyl Alcohol had full ribs on C7 (7%), C5 with tubercula anterior (3%), and split and/or incomplete C2 (left, 10%; right, 15%). The observations suggested posteriorization of the C2–C7 vertebrae. Foramina and other unspecified characteristics were observed on vertebrae, anterior to where they normally occur. Other signs of developmental toxicity were: >8 attached ribs, split and/or incomplete C1, offset sternum, C7 fused to T1, and < or >26 presacral vertebrae. These lesions had "clear dose responses," but the incidences were not reported.

Embryo Culture

Sprague-Dawley rat embryos exposed to 8 to 16 mg/ml Methyl Alcohol had dose-related decreases in somite number, head length, and developmental score. The 12-mg/ml dose was embryolethal, and caused dysmorphogenesis; the high dose was also embryolethal. When CD-1 mouse embryos were treated with 2 to 8 mg/ml Methyl Alcohol, all but the lowest dose caused significant decreases in crown-rump length, and the embryos of the high-dose group had 80% embryolethality (Andrews et al. 1993).

A second whole-embryo culture study indicated that Methyl Alcohol (8–16 mg/ml; 0.25–0.499 M) caused increased cell death in specific regions of both Sprague-Dawley rat and CD-1 mouse embryos, including the forebrain, visceral arches, and the otic and optic placodes. Derivatives of these regions manifested morphological abnormalities after in vivo exposure. Exposure to Methyl Alcohol did not increase cell death in neuroepithelium or neural folds. Neural tube defects were not explained by excess cell death. The results suggested that increased cell death in specific regions of the exposed embryos had a role in producing defects, which included cleft palate and malformations of the cranium, eyes, and ears (Abbott, Ebron-McCoy, and Andrews 1995).

CD-1 mouse embryos were treated with 6 to 20 mg/ml Methyl Alcohol in serum-free organ culture. The craniofacial explants were then examined for alterations morphology, fusion, proliferation, and growth. The medial epithelium was either degenerated completely or remained intact in unfused palates; the severity of these changes was dose-dependent, and both changes interfered with fusion. Cellular proliferation was a specific and sensitive target for Methyl Alcohol. The craniofacial tissues had reduced total DNA content at exposures that did not affect total protein content. Both DNA and protein decreased with increased exposure to Methyl Alcohol, however (Abbott, Logsdon, and Wilke 1994).

Ebron-McCoy et al. (1994) investigated the developmental toxicity of formate and formic acid in mouse and rat whole embryo culture. Day 9 rat embryos were exposed for 24 or 48 hours, and day 8 mouse embryos were exposed for 24 hours to 0.2 to 3.0 mg/ml formate or formic acid. The indices of growth and development were decreased in mouse and rat embryos treated with formate for 24 hours, and the number of abnormalities were increased at the larger doses. Embryo lethality did not increase. Rat embryos exposed to 0.2 to 2.0 mg/ml formate for 48 hours had dose-related decreases in the growth and development parameters. At the larger doses, embryo lethality and the number of abnormal embryos were increased. Mouse and rat embryos exposed to formic acid for 24 hours and rat embryos exposed for 48 hours had decreased growth and development, and increased numbers of dead and abnormal embryos. The investigators concluded that exposure to formate or formic acid at comparable times during development was embryotoxic for both species at concentrations $\geq$4-fold lower than those for Methyl Alcohol.

The effects of Methyl Alcohol and formic acid on the embryonic development of the rat were performed in vitro using rat embryo culture. Methyl Alcohol and formic acid had dose-dependent embryotoxic effects. The NOEL of Methyl Alcohol was 211.7 μmol/ml, and embryotoxicity was observed at 286.5 μmol/ml. The NOEL of formic acid was 3.74 μmol/ml, and severe embryotoxicity was observed at 18.6 μmol/ml. Both low pH and formate contributed to the observed embryotoxicity of formic acid (Brown-Woodman et al. 1995).

ROLE OF METABOLITES IN METHYL ALCOHOL-INDUCED TOXICITY

Historically, Methyl Alcohol toxicity was attributed to impurities such as acetone. After those impurities were eliminated by newer manufacturing techniques, toxicity was attributed to Methyl Alcohol itself, lactic acid, and formaldehyde (Wood and Buller 1904; Wood 1906).

By 1943, however, the metabolism of formaldehyde to formic acid and formate following exposure to Methyl Alcohol was well known (Röe 1943). Further research suggested that the toxicity of Methyl Alcohol was due to its metabolism to formic acid and formate. Formate accumulation appeared to be responsible for the generation of metabolic acidosis in the monkey and human during early toxicosis. Nonprimate species such as the rat did not normally accumulate formate and, therefore, did not have signs of metabolic acidosis. Rats and other nonprimates were able to metabolize formate to CO_2 more quickly (by ~50%) than monkeys and humans. Whereas Methyl Alcohol distributed uniformly throughout the body, the metabolites did not. Metabolites generated in situ within a particular organ or tissue had different toxic potentials than the same substances administered parenterally or enterally (Tephly and McMartin 1984).

In order to determine whether formaldehyde accumulated during Methyl Alcohol exposure, McMartin et al. (1979) administered either a 20% Methyl Alcohol solution or a [^{14}C]Methyl Alcohol solution via a nasogastric tube to young male and female cynomolgus monkeys. Five monkeys were used in this study (Table 7). Plasma bicarbonate values were calculated from arterial pH and pCO_2 values. Samples of arterial blood, urine, cerebrospinal fluid, and the liver were obtained and analyzed for formate after the signs of toxicity were apparent. The monkeys were killed, and samples of the anterior and posterior optic nerves, anterior optic disc, kidneys, cerebrum, and midbrain were obtained. Homogenates (25%) were prepared from the tissue samples and analyzed for formate.

The researchers evaluated formaldehyde metabolism using the dimedon assay and the chromatropic acid method. Both the femoral vein and femoral artery of the same leg were cannulated, and [^{14}C]formaldehyde was infused intravenously as a dose of 1 mmol/kg over 3 to 4 minutes. Arterial blood samples were withdrawn and analyzed for formate, formaldehyde, pH, and pCO_2.

TABLE 7
Metabolite analysis using Cynomolgus monkeys (McMartin et al. 1979)

Monkey	Treatment	Diet	Procedure
A	2 g/kg Methyl Alcohol, 20% solution orally	Folate-deficient (FD)	Chromatropic acid assay
B	3 g/kg Methyl Alcohol, 20% solution orally	Control	Chromatropic acid assay
C	3 g/kg [^{14}C]Methyl Alcohol, 20% solution orally (specific activity 1300 dpm/μmol)	Control	Dimedon assay
D	Infusion of 1 mmol/kg [^{14}C]formaldehyde, 0.2 M solution in 0.9% saline (specific activity 1500 dpm/μmol)	Control	Dimedon assay
E	Infusion of 1 mmol/kg [^{14}C]formaldehyde, 0.2 M solution in 0.9% saline (specific activity 115,000 dpm/μmol)	Control	Dimedon assay

No formaldehyde accumulated in the blood of either the Methyl Alcohol–treated control monkeys (B and C) given 3 g/kg of the alcohol, or in the blood of a Methyl Alcohol–treated, FD monkey (A) given 2 g/kg during the time when marked acidosis was observed. Formate concentrations in the blood were elevated, but the formate concentrations in the FD monkey were approximately twice those observed in monkeys of the control group. The monkeys had signs and lesions of Methyl Alcohol poisoning, including optic disc edema and vomiting.

Formaldehyde was not detected in the cerebrospinal fluid, vitreous humor, or urine of treated monkeys, or in tissues of the liver, kidneys, optic nerve, cerebrum, or midbrain. The formate concentration in the urine was approximately 10-fold greater than that in the blood, which indicated that formate was concentrated by the kidneys. Formate concentrations were markedly increased in the vitreous humor, but not as much as in the blood. The concentration of formate in the cerebrospinal fluid of one monkey was about one-half the blood concentration. The monkey (E) given the [^{14}C]formaldehyde with the high specific activity had a short episode of vomiting, but no other signs of toxicity were observed. Formaldehyde was detected for at least 60 minutes, and the half-life for formaldehyde elimination from the blood was ~1.5 minutes. In the other monkey given the radiolabel (D), formaldehyde was detected for ~5 minutes after the infusion was completed. The researchers concluded that formaldehyde was not a major factor in the toxicity induced by Methyl Alcohol in the monkey (McMartin et al. 1979).

In similar studies using male and female cynomolgus monkeys, Noker and Tephly (1980) administered 2 g/kg [^{14}C]Methyl Alcohol (20% in water) via a nasogastric tube. The monkeys were placed immediately in a metabolic chamber. Blood samples were obtained and expired [^{14}C]Methyl Alcohol and [^{14}C]CO_2 were collected at timed intervals. Blood concentrations of Methyl Alcohol, blood gases, and blood pH were determined. An indwelling catheter was implanted into the femoral vein and/or artery of each monkey for Methyl Alcohol metabolic studies. For formate metabolism studies, sodium [^{14}C]formate (2.5 M sodium formate in water; specific activity 12,000 dpm/mg) was administered IP, and expired CO_2 was collected and analyzed for radioactivity. Liver samples were obtained and analyzed for folate after treatment with hog kidney conjugase, using the test organisms *Lactobacillus casei* (ATCC 7469) and *Pediococcus cerevisiae* (ATCC 8081) in bacto–folic acid casei medium. Some of the monkeys were also treated with multiple doses of 200 μg/kg 5-THF (as its calcium salt; 1% in saline), IV or IP, at 48, 24, and 1 hour prior to sodium [^{14}C]formate infusion.

The concentration of total folates in the normal monkey liver was significantly smaller than the concentration in the rat liver. In the monkey, the severity of Methyl Alcohol toxicity and the accumulation of formate in the blood was reduced by treatment with repetitive doses of 5-THF. Formate accumulation was decreased by 50%, compared to that of monkeys given Methyl Alcohol alone. In addition, monkeys given 5-THF did not develop metabolic acidosis and bicarbonate depletion. Methyl Alcohol toxicity was also alleviated when 5-THF was administered after signs of toxicity had developed. The rate of Methyl Alcohol elimination from the blood did not change after treatment with 5-THF, but the rate of CO_2 production via formate oxidation increased from 22.2 ± 0.4 to 28.9 ± 0.6 mg/kg/h. The investigators concluded that a reciprocal relationship existed between formate accumulation and depletion of blood bicarbonate (Noker and Tephly 1980).

The toxic effects of formic acid were due to the inhibition of cytochrome oxidase complex (an integral protein complex of the inner mitochondrial membrane) at the terminal end of the mitochondrial electron transport chain, which lead to "histotoxic hypoxia." Formic acid bound the sixth coordination position of ferric heme iron to inhibit the cytochrome oxidase complex; the K_i was 1 mmol. Acidosis caused by the increase of undissociated formic acid potentiated the inhibition of cellular respiration, thus hastening cellular damage (Liesivuori and Savolainen 1991). Nicholls (1976) reported that formate inhibited cytochrome *c* oxidase activity both in intact mitochondria and submitochondrial particles, and in isolated cytochrome aa_3. Inhibition increased with decreased pH, indicating that formic acid (HCOOH) could have been the inhibitory species. The K_i for formate inhibition of cellular respiration was a function of the reduction state of the system, and varied from 30 mM (100%

reduction) to 1 mM (100% oxidation) at pH 7.4 and 30°C. In addition, formate inhibited succinate–cytochrome *c* reductase activity.

Infurna and Berg (1982) investigated the distribution and metabolites of Methyl Alcohol in maternal and fetal tissues. Gravid rats were exposed to 2% Methyl Alcohol in drinking water, and were killed on the following day after approximately 2 g/kg had been consumed. [^{14}C]-Methyl Alcohol was administered either overnight in the same water, or by gavage 1 hour before the rats were killed. One hour after the radioactive compound was administered, Methyl Alcohol and metabolite concentrations averaged 45 μmol/g in maternal tissues, 50 μmol/g in fetal bodies and livers, and 55 μmol/g in fetal heads and brains. After an overnight exposure to the compound, the average concentrations were 20 μmol/g in maternal tissues, 25 μmol/g in fetal tissues, and 30 μmol/g in fetal brains. Formate constituted 20% of the radioactivity (average) extracted from maternal livers. In fetal livers, the average was 3%, and it more than doubled overnight. The averages in maternal kidneys were 10% in 1 hour, and 1% overnight. The results suggested a rapid equilibrium of Methyl Alcohol across placental and blood-brain barriers, with more active production of formate in adult livers than in other tissues.

After Methyl Alcohol poisoning in humans, the accumulation of formic acid increased the observed acidosis (McMartin, Ambre, and Tephly 1980). Two patients, a 63-year-old male (patient 1) and a 50-year-old male (patient 2), were admitted to the hospital in a comatose state with fixed and dilated pupils. At the time of admission, patient 2 had a blurred, edematous optic disc. Arterial blood samples of both men had alterations indicative of severe metabolic acidosis and the presence of high concentrations of Methyl Alcohol. Treatment of the acidosis was immediately with sodium bicarbonate and IV infusion of 10% ethyl alcohol to retard Methyl Alcohol metabolism; hemodialysis was used to increase the elimination of Methyl Alcohol. Blood samples were obtained during treatment and were analyzed for blood concentrations of formate and Methyl Alcohol. Patient 1 died without regaining consciousness after respiratory arrest and cardiovascular collapse. Patient 2 regained consciousness in the late stages of dialysis and survived without apparent permanent sequelae. During treatment, peak Methyl Alcohol concentrations in the blood were 104 and 185 mg/100 ml for the two patients, respectively. The initial formate concentration in patient 2 at 4 hours after admission was 11.1 mEq/l. In patient 1, the initial formate concentration at 8 hours after admission was 26 mEq/l. The plasma bicarbonate concentration was less than 3 mEq/l in both patients; the normal content is 22 to 28 mEq/l. The observed increase of the anion gap was considered caused by the decrease in plasma bicarbonate. Sixty percent to 100% of the decrease in plasma bicarbonate appeared to have been replaced with formate. Hemodialysis rapidly decreased the blood concentrations of Methyl Alcohol and formic acid; the half-lives for Methyl Alcohol elimination were 6 and 4 hours for patients 1 and 2, respectively. The formate concentrations decreased rapidly (half-lives of 1–2 hours). The urine of patient 2 had large amounts of formic acid (42.1 mEq/l) during the first 34 hours of treatment; after 34 hours, the concentration was 0.43 mEq/l. The formate urinary concentration was greater than any observed in the blood, which indicated concentration by the kidneys. The urinary concentration of Methyl Alcohol was 63.7 mEq/l during the first 34 hours, and 1.2 mEq/l after 34 hours. After 34 hours, no increase was observed in blood formate concentration.

In order to determine if formate was a sensitive biological marker of Methyl Alcohol toxicity or exposure, 26 human subjects were exposed to 200 ppm of Methyl Alcohol vapors for 4 hours. Concentrations of endogenous serum formate and urinary formate were measured. Each volunteer served as his or her own control, and participated in both sham and Methyl Alcohol exposures. Urine and serum specimens were collected before, during, and after exposure to Methyl Alcohol vapors. No significant differences were observed in serum formate concentration between exposed and control humans were observed. The mean concentrations were 14.28 ± 8.90 (exposed) and 12.68 ± 6.43 (control) mg/l. At 4 hours, a slight, but nonsignificant, increase in urinary excretion of formate was observed. The mean excretion rates were 2.7 ± 1.69 (exposed) and 1.67 ± 1.02 (control) mg/4 h. The investigators concluded that serum and urine formate determinations were not sensitive markers of Methyl Alcohol exposure at the threshold limit value (TLV) (d'Alessandro et al. 1994).

EFFECTS OF ETHYL ALCOHOL ON METHYL ALCOHOL–INDUCED TOXICITY

Methyl Alcohol is apparently oxidized in the body at less than one fifth of the rate of ethyl alcohol (Bennett et al. 1953). Ethyl alcohol has a 10 to 20 times greater affinity for hepatic alcohol dehydrogenase, and can reduce the rate of Methyl Alcohol metabolism by approximately 90% via competitive inhibition (Zatman 1946; Kendal and Ramanathan 1952). As a result, Methyl Alcohol is oxidized 10 times more slowly than ethyl alcohol (Ellenhorn and Barcelous 1988). At the same time, administration of ethyl alcohol increases urinary excretion of Methyl Alcohol by approximately 1% to 4%, hence the use of ethyl alcohol for the treatment of Methyl Alcohol intoxication (Downie et al. 1992).

Urinary excretion of formate was increased when Methyl Alcohol was adminstered to a dog simultaneously with ethyl alcohol, amyl alcohol, or acetone (Leaf and Zatman 1952; McMartin, Ambre, and Tephly 1980). Kendal and Ramanathan (1953) reported, however, that the appearace of formate in the urine of humans was completely inhibited when repeated doses of ethyl alcohol were administered. Formate appeared in the urine 1 to 2 hours after the cessation of ethyl alcohol administration, and was coincident with the rapid decrease of body Methyl Alcohol concentration which then ensued.

Symptoms were milder in patients who consumed ethyl alcohol at the same time as Methyl Alcohol (Röe 1943; Bennett et al.

1953). Both alcohols are oxidized by catalase in the presence of hydrogen peroxide; in studies of the inhibitory effect of anions upon catalase activity, formic acid was 800 times stronger in its catalase inhibitory action than acetic acid. In simultaneous injection studies using rabbits, Methyl Alcohol was unchanged in the blood until the dose of ethyl alcohol had been oxidized (Keilin and Hartree 1945; Agner and Belfrage 1947; Bennett et al. 1953; Röe 1955). Zatman (1946) demonstrated that alcohol dehydrogenase oxidized Methyl Alcohol at one-ninth the rate for ethyl alcohol, and that ethyl alcohol in equimolar concentrations completely inhibited the oxidation of Methyl Alcohol. Inhibition also occurred with molar ratios of ethyl alcohol as great as 1:16. If most of the Methyl Alcohol had been metabolized, however, treatment with ethyl alcohol could exacerbate the acidosis (Buckley and Vale 1986). The excretion of formate in animals decreased when simultaneous doses of ethyl alcohol were administered with Methyl Alcohol (Browning 1965).

The serum half-life of Methyl Alcohol after mild toxicity was 14 to 20 hours, and after severe toxicity, 24 to 30 hours. Treatment with ethyl alcohol increased the serum half-life to 30 to 35 hours (Ellenhorn and Barcelous 1988). Other investigators noted that ethyl alcohol increased the toxicity of Methyl Alcohol in experimental animals such as mice, but this was likely due to the combined anesthetic effect of the two alcohols (ethyl alcohol has an anesthetic potency of approximately twice as much as Methyl Alcohol). In humans, treatment with ethyl alcohol can prevent a recurrence of acidosis during alkali treatment, compared to treatment with alkali alone (Röe 1955).

MUTAGENICITY

Hayes et al. (1990) investigated the mutagenicity of several low molecular weight alcohols, including Methyl Alcohol, using a short-term forward mutagenesis assay, the RK^+ mutatest. In this assay, a temperature-sensitive repressor gene from bacteriophage λ was integrated as a nonexcisable DNA segment into the chromosome of *Escherichia coli* strain SA500(λ*c*I857Δ431). *E. coli* cell viability was controlled by the repressor, which was necessary for the initiation and regulation of λ replication. When the incubation temperature was increased from 30° to 42°C, bidirectional replication forks were initiated from the λ DNA segment, which proceeded onto the *E. coli* chromosome, killing the cells. Thus, these cells had an RK^+ replicative killing competent phenotype. (The frequency of spontaneous mutation from RK^+ to RK^- was between 0.5 and 1.5×10^{-7}.) In previous studies, ethyl alcohol stimulated the appearance of RK^- mutations in RK^+ mutatest cells. In this study, several alcohols, including Methyl Alcohol, had mutagenic effects similar to that of ethyl alcohol.

Overnight tryptone broth cultures were derived from a single colony of an RK^+ strain of *E. coli*. Untreated control cell cultures had 10- or 20-fold concentrated RK^+ cells (0.25 ml) and 0.25 ml buffer (0.1 M NaCl, 0.01 M TRIS · HCl; pH = 7.5). Parallel cell treatment mixtures (0.5 ml final volume) were made with 0.25-ml aliquots of the same cells, buffer, and alcohol. The cultures were incubated at 30°C for 10 minutes and centrifuged at 15000 × *g* for 1 minute. The cells were resuspended in 0.5 ml buffer and were plated at 42°C on tryptone broth agar. The positive controls were *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (with 10% DMSO) and ^{60}Co ionizing radiation.

Methyl Alcohol induced mutations that suppressed the lethal loss of replication control from a prophage fragment of bacteriophage λ. The threshold concentration for toxicity and mutagenicity for strain SA500(λ*c*I857Δ431) was 23% Methyl Alcohol (Figure 1, Table 8). For strain SA500(λ*bio*257*c*I857

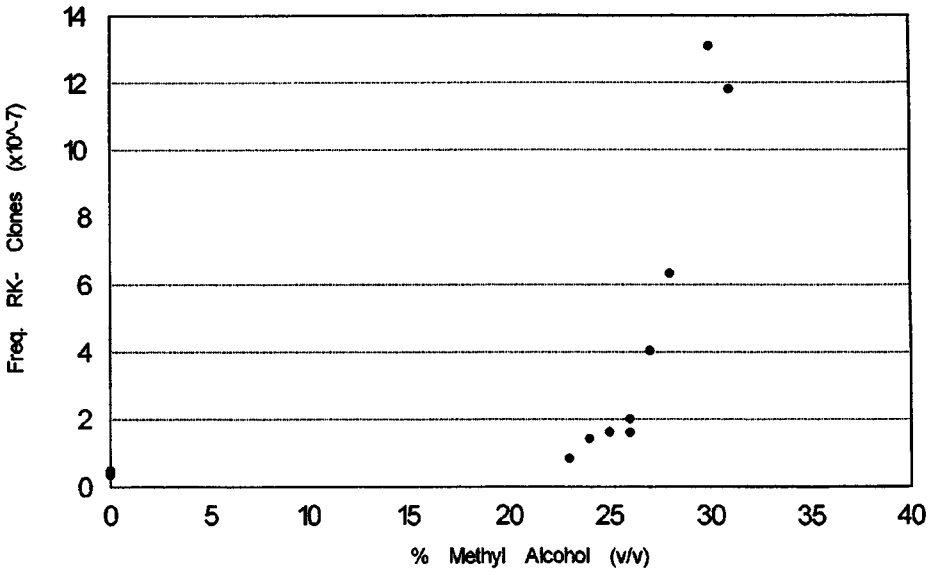

FIGURE 1
RK+ Mutatest (Hayes et al. 1990).

TABLE 8
Forward mutations in *E. Coli* RK^+ Strain SA500(λcI857Δ431) (Hayes et al. 1990)

Treatment	% Relative Survival[a]	Frequency of RK^- Clones ($\times 10^{-7}$)[b]	Mutation index[c]
Methanol (% *v*/*v*)			
Group 1			
0	100	0.48	1.0
23	53	0.83	1.7
24	38	1.41	2.9
25	29	1.61	3.4
26	24	2.00	4.2
Group 2			
0	100	0.35	1.0
26	22	1.60	4.6
27	7	4.02	11.5
28	3.5	6.32	18.1
Group 3			
0	100	0.41	1.0
29	0.11	71.43	174.2
30	0.04	11.08	31.9
31	0.02	11.81	28.8
$^{60}CO_2$ irradiation (positive control)			
0	100	1.06	1.0
9.7 centigrey/min	33	9.00	8.5
N-methyl-*N'*-Nitro-*N*-Nitrosoguanidine (positive control)			
0 (dilution buffer)	100	1.33	1.0
0 (10% DMSO)	83	1.26	0.9
25 μg/ml	46	120.00	90.2

[a]Treated cell titer × 100/untreated cell titer; at 30°C.
[b]Average number of RK^- survivor clones at 42°C/cell titer at 30°C.
[c]Mutation frequency/spontaneous mutation frequency.

Δ431), concentrations of 26% to 31% Methyl Alcohol did not increase the mutation index greater than 1.6, but greater concentrations of Methyl Alcohol were significantly toxic to the cultured cells.

Fritzenschaf et al. (1993) tested Methyl Alcohol and other chemicals using the Syrian hamster embryo micronucleus test (in vitro) and correlated the results with those of the in vivo micronucleus formation assay and the Syrian hamster embryo cell transformation assay. In all three assays, Methyl Alcohol was not mutagenic (Fritzenschaf et al. 1993).

Methyl Alcohol was nonmutagenic in the *E. coli* SOS chromotest and the *Salmonella*/mammalian microsome mutagenicity assay. These assays used strains PQ37 (*E. coli*), TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, and/or TA1538 (*S. typhimurium*), with and without S9 metabolic activation (Gocke et al. 1981; Mersch-Sundermann et al. 1994). In an assay using the yeast *Schizosaccharomyces pombe*, Methyl Alcohol was not mutagenic with and without S9 metabolic activation (Abbondandolo et al. 1980). Methyl Alcohol did not induce chromosomal aberrations in the root-tip meristem of *Vicia faba* (Obe and Ristow 1979). In other studies, Methyl Alcohol did not cause mutations in the Basc test on *Drosophila* (detecting sex-linked recessive lethal mutations), or in the micronucleus test using NMRI mouse bone marrow cells (Gocke et al. 1981). O'Loughlin et al. (1992) found no indication of genotoxic potential using an erythrocyte micronucleus assay when folic acid–deficient and normal Swiss mice (10 per group) were exposed to up to 2500 mg/kg of Methyl Alcohol four times daily in feed.

The metabolite formaldehyde induced sister chromatid exchanges in a positive dose-dependency in Chinese hamster ovary cells (0.1–0.4 $\times 10^{-3}$% doses), and in human lymphocytes (0.1–1 $\times 10^{-3}$% doses). Formaldehyde also induced crosslinks and single-strand breaks in DNA, and was mutagenic in *Drosophila* (Obe and Ristow 1979).

CLINICAL ASSESSMENT OF SAFETY

Acute Toxicity

The toxicity for humans of Methyl Alcohol has been well documented in the published literature (Wood and Buller 1904;

Bennett et al. 1953; Sharpe et al. 1982; Smith 1983; Jacobsen and McMartin 1986; Kavet and Nauss 1990; Von Burg 1994). Nearly a century ago, Wood and Buller (1904) published a series of 235 case studies that characterized the features of acute Methyl Alcohol poisoning. In most cases, the patients had ingested Methyl Alcohol as a substitute for alcoholic beverages, and often became blind and/or died. In several cases, patients claimed exposure via skin contact and inhalation, but they were often not believed by authorities, who thought the patients had drunk the alcohol and subsequently lied to doctors.

Methyl Alcohol is considered to be highly toxic, and it can cause severe metabolic acidosis, blindness, and death. Methyl Alcohol toxicity is manifested earlier and at a lower dose, as compared to the toxicity of ethyl alcohol, but the comparative dose for end point toxicity (death) is the same for both alcohols (Von Burg 1994). As Methyl Alcohol distributes readily and uniformly throughout all tissues and organs, all routes of exposure to the alcohol are "toxicologically equivalent" (Kavet and Nauss 1990). In general, serum concentrations of Methyl Alcohol do not correlate with the clinical condition, but serum concentrations of formate do correlate (Swartz, Millman, and Billi 1981; Osterloh et al. 1986).

Methyl Alcohol intoxication causes the marked depletion of bicarbonate, and plasma bicarbonate concentrations as low as 4.0 mEq/l (blood pH $\geq$6.9) have been reported. The concurrent decrease in blood pH and blood pCO_2 indicated that acidosis caused by exposure to Methyl Alcohol was an uncompensated metabolic acidosis (Tephly and McMartin 1984). Currently, the present treatment for Methyl Alcohol poisoning involves treatment with ethyl alcohol and hemodialysis, although alkali treatment can be used to normalize blood pH (Jacobsen and McMartin 1986; Jacobsen et al. 1988).

Six healthy human volunteers exposed at rest or during exercise to 200 ppm Methyl Alcohol vapors for 6 hours or who were exposed to 20 mg/kg Methyl Alcohol orally had elevated blood concentrations of Methyl Alcohol, but blood formate concentrations were not significantly increased above endogenous concentrations. The researchers stated that "human populations may not be at added risk of neurotoxic effects resulting from exposure to low levels of methanol" (Lee et al. 1992). Researchers suggested that human populations with dietary folate deficiency (e.g., pregnant women or the elderly) could be more sensitive to the effects of Methyl Alcohol (Lee et al. 1992; Medinsky and Dorman 1993, 1995).

Oral

The majority of Methyl Alcohol exposures reportedly resulted from the accidental or intentional ingestion of adulterated beverages or Methyl Alcohol products (Kavet and Nauss 1990). Individual susceptibility to the acute toxicity of Methyl Alcohol varied. The ingestion of 4 ml of absolute Methyl Alcohol by humans caused blindness in some cases. The ingestion of 15 ml of a 40% solution caused death in one case, whereas the ingestion of 500 ml of a 40% solution was not fatal in another. Typically, the ingestion of 80 to 150 ml of 80% proof Methyl Alcohol was fatal.

Symptoms of Methyl Alcohol intoxication (after ingestion) were delayed for 12 to 18 hours, during which time the only clinical sign was a state of mild inebriation. Afterwards, the symptoms included headache, anorexia, weakness, fatigue, leg cramps, and/or pain and vertigo. Severe gastrointestinal pain, nausea, vomiting, and diarrhea sometimes occurred. Rarely, patients had excitement, mania, or convulsions. Patients complained of dimness of vision and had dilated pupils that reacted poorly to light, followed by bilateral blindness. The onset of acidosis coincided with breathlessness (rapid and shallow breathing) and tachypnea. CNS infarcts in the basal ganglia (putamen) have been reported, as well as parietal and cerebellar cortical degeneration and necrosis (Von Burg 1994).

Methyl Alcohol poisoning has been implicated as a precipitant for Parkinson's disease (Ley and Gali 1983; LeWitt and Martin 1988; Von Burg 1994). LeWitt and Martin (1988) reported a case report of a 30-year-old male who ingested an unknown quantity of windshield washing fluid (35% to 98% Methyl Alcohol). The only neurological outcome immediately at treatment was bradykinetic dystonia. Six years after the Methyl Alcohol intoxication, a computed axial tomography (CAT) scan uncovered bilateral putaminal cystic lesions. In another case, a 40-year-old woman was poisoned with 250 ml of a denatured alcohol preparation that contained Methyl Alcohol (Ley and Gali 1983). She had signs and symptoms of Parkinson's disease, including tremors, hypokinesis, muscular rigidity, and stuttering speech. In a CAT scan performed 45 days after admission, symmetrical areas of decreased attenuation were found at the lenticulocapsular regions. These areas persisted without changes for 4 months after the poisoning occurred; mild rigidity, tremor, and hypokinesis persisted as sequelae.

Sudden cardiac failure was also a sign associated with Methyl Alcohol intoxication (Bennett et al. 1953; Cavalli et al. 1987). Chronic exposure to Methyl Alcohol caused lesions of edema, granular degeneration, and necrosis of heart muscle fibers, as well as fatty degeneration of the heart muscle (Browning 1965). In the liver, parenchymatous degeneration, focal necrosis, and fatty infiltration were observed. The kidneys had congestion and parenchymatous degeneration, and the spleen often was dark blue. The lungs had various inflammatory lesions, as well as edema, congestion, and desquamation of alveolar epithelium. In fatal cases, terminal pneumonic consolidation was noted. In the nervous system, capillary congestion, edema, and patchy degeneration of the neurons were observed, as well as more severe congestive lesions.

A single outbreak of Methyl Alcohol poisoning involved 97 patients, of which 28 died. The latent period between consumption of the adulterated drink containing the alcohol and the development of symptoms ranged from 7 1/2 to 60 hours. Of

the 97 cases, 75% had CNS symptoms, 89% had shock either at admission or as a late event, 42.8% had blurred vision, and 10.7% were blind. The earliest death occurred at 7 1/2 hours, and the last at 12 days. Methyl Alcohol concentrations in the blood and viscera varied from 155.8% to 420.4 mg%. The 28 fatal cases (21 males, 7 females) were autopsied. In four cases, a significant concentration of Methyl Alcohol was detected in the stomach contents 5 to 12 days after consumption. Shrinkage and degeneration of neurons in the parietal cortex were observed in 85.7% of the fatal cases. The temporal cortex (28.5%), cerebellar granular layer (32.1%), and Purkinje cell (28.5%) were also affected. Putamental degeneration and necrosis (7.14%), hemorrhage (3.5%) and sponginess (7.14%) of the optic chiasma, and fatty change (67.8%) and microvesicular fat (42.5%) of the hepatocytes were observed. Renal changes included severe renal tubular degeneration and patchy necrosis (100%), severe congestion of peritubular capillaries (71.42%), moderate glomerular capillary dilatation and congestion (78.57%), mild endothelial swelling (35.7%), and mild mesangial proliferation (35.7%). Other lesions included mild pulmonary edema (64.28%), cloudy swelling in the myocardium (42.5%), congestion and patchy hemorrhage of the gastric mucosa (21.3%), and acute pancreatitis (3.5%) (Mittal, Desai, and Khade 1990).

An additional 323 cases of Methyl Alcohol poisoning due to the ingestion of adulterated whisky were reported by Bennett et al. (1953). Of the patients, 210 were men and 113 were women. Forty-one were dead upon arrival at the hospital or died within 30 minutes of arrival. Another 19 died after that period. The whisky was analyzed and found to contain 35% to 40% Methyl Alcohol. Benton and Calhoun (1952) investigated the ocular effects observed during this episode. Visual symptoms occurred 18 to 48 hours after ingestion of the whiskey and included blurred vision, loss of central vision, and complete blindness. The pupils reacted "sluggishly or not at all to light." Visible retinal changes were hyperemia of the optic nerve head, superficial edema of the papilla and surrounding retina, and atrophy of the optic nerve. All patients with severe retinal edema had some permanent vision loss. The degree of acidosis correlated with the severity of general and ocular symptoms.

A 44-year-old male who died as a result of Methyl Alcohol intoxication (the amount consumed was not available) had a recent ischemic infarct of the tip of the right temporal lobe, severe fatty changes of the liver, and hypertrophy of the muscle of the left ventricle of the heart. At microscopic examination, pallor of the myelin of the retrolaminar optic nerves, bronchopneumonia, and pulmonary edema were observed. The Methyl Alcohol concentration of the organs, gastric contents, urine, bile, blood, vitreous humor, and other tissues ranged from 93 to 175 mg/100 g (Wu Chen, Donoghue, and Schaffer 1985).

Percutaneous

Cases of percutaneous absorption of Methyl Alcohol are relatively rare due to the alcohol's high volatility and rapid evaporation rate. If absorbed through the skin, Methyl Alcohol is distributed through the water compartment of all tissues and organs (see "General Biology—Absorption, Metabolism, Distribution, and Excretion"). Two percent to 12% of the absorbed alcohol is eliminated unchanged from the kidneys and lungs, but the remainder is primarily metabolized in the liver (Ellenhorn and Barcelous 1988; Kavet and Nauss 1990; Aufderheide et al. 1993). McCord (1931) suggested that, based upon results using rhesus monkeys (see "Animal Toxicology—Acute Toxicity"), the absorption of 31 ml of Methyl Alcohol in 41 hours (18 g/24 h) is dangerous to humans.

Downie et al. (1992) reported a case of percutaneous industrial toxicity of Methyl Alcohol. A tanker that had previously carried palm oil was washed down with Methyl Alcohol prior to being filled with Methyl Alcohol for transport. A 31-year-old crewman with a mild sunburn on his trunk and arms remained in the 950-m^3 tank for 2 to 3 hours while the bottom of the tank was flooded with Methyl Alcohol, and a second crewman used a pump to spray the walls. Both crewmen wore breathing apparatus, and the latter also wore polyvinylchloride (PVC) protective suiting. The former did not wear the suiting due to his sunburn, and instead wore a shirt, shorts, and sandals. At the end of the procedure, the unprotected crewman noticed that his clothes were soaking, and his feet were wet. He then worked on deck for approximately 1 hour until his clothing dried.

The crewman felt lethargic at that time, and retired to bed. He awakened feeling ill, and had blurred vision, painful eyes, and dizziness. He also had severe acid dyspepsia. A doctor examined him and prescribed eye drops, antacid, and bed rest. Two hours later, the Methyl Alcohol company plant manager visited him and recognized the symptoms of Methyl Alcohol toxicity. The patient was admitted to a hospital with severe acidosis, and was treated with IV bicarbonate and oral whisky. The optic discs were red and blurred. A blood Methyl Alcohol estimation was not performed, and he was treated with IV 10% ethyl alcohol and bicarbonate, and oral folic acid. He became semicomatose later that day. Two days later, an ophthalmologist reported crusted optic nerve heads with blurred margins, but no hemorrhages, exudates, or edema. The patient's vision returned to normal by the third day, and the treatment with IV ethyl alcohol was discontinued (Downie et al. 1992).

Kahn and Blum (1979) reported a case of an 8-month-old male who died after warm compresses soaked with Methyl Alcohol were applied to his chest for approximately 12 hours per night for 2 nights prior to admission. The parents told doctors that the child had a cough and low-grade fever, and it was their traditional treatment to rub the child's chest with olive oil before applying ethyl alcohol–soaked pads to the chest overnight. The mother accidentally purchased Methyl Alcohol, which was used instead. The child was also given acetylsalicylic acid.

The day before admission, the child had progressively rapid and shallow respiration. A radiograph of the chest was considered normal. The child had increasing drowsiness during the afternoon, and did not wake up for his evening meal. The day of admission, the child had worsened dyspnea, and was in a coma

upon admission. The pupils were dilated and unresponsive to light; both fundi had recent hemorrhages, and slight papillary edema was observed. Tendon reflexes were absent, and slight cyanosis and some petechiae on the thorax were observed. The child was treated with artificial ventilation and antibiotics; later, bicarbonate was added due to the signs of severe metabolic acidosis. Blood and urine analyses confirmed generalized hyperaminoacidemia and exaggerated excretion of methionine and proline in urine, as observed during acute liver dysfunction. Toxicologic screening at the time of admission was negative. Samples of blood and urine were sent to a poison control center laboratory, which confirmed the presence of 40 ml/dl Methyl Alcohol in the blood. The child was then treated initially with 0.33 g/kg ethyl alcohol by IV (then with 0.26 g/kg every 4 hours) and peritoneal dialysis (the dialyzing fluid contained sodium bicarbonate instead of the usual sodium lactate). Hemodialysis was not instituted due to the development of hemodynamic instability despite the early administration of dopamine. Methyl Alcohol was identified in the urine and peritoneal fluid, but no quantitative estimation was determined. The child's condition deteriorated rapidly, accompanied by hypothermia and episodes of bradycardia. The arterial blood pressure fell in the evening, and the child died shortly thereafter. The liver at the time of death had diffuse fatty degeneration; the lumbar spinal fluid had blood (Kahn and Blum 1979).

Similar cases were reported by Giménez et al. (1968). Nineteen children (12 girls, 9 boys; aged 1.5 months to 4 years) were treated using Methyl Alcohol–soaked pads by their parents for cramps, pain, vomiting, diarrhea, and/or crying, excitability, and irritability. The pads were applied overnight to the abdominal region (under rubber panties) for up to 3 nights. Two additional children were treated with pads soaked in both Methyl Alcohol and ethyl alcohol. Half of the children had marked malnutrition. The asymptomatic period between time of application and onset of symptoms lasted from 1 to 13 hours, and the time between application and diagnosis was 4 to 48 hours.

Early signs of toxicity were CNS depression of variable degree and alternate periods of excitation. Thirteen children had severe respiratory depression, and others had hyperpnea and typical respiratory rates. Eleven children had generalized tonic-clonic convulsions with hypertonic or absent deep tendon reflexes. Miosis was observed in 12 cases, and mydriasis in nine. Nine children had recurrent cardiac arrests. Seven children had anuria or severe oliguria. Five patients that had severe acidosis and hyperkalemia also had abdominal skin lesions; three were erythematous, and two were scaling. Two children had papillary edema and ocular fundus bleeding.

Methyl Alcohol blood concentrations were 0.057% to 1.13 g% (in 11 children). The pH values were 6.4 to 7.38 upon admission, and blood sugar concentrations varied from 0.0% to 130 mg%. The children were treated with 3.75% sodium bicarbonate IV, and were given glucose and ethyl alcohol. Two of the children also received exchange transfusion, and 14 received peritoneal dialysis. Twelve of the 19 children given Methyl Alcohol alone died within 2 to 10 days after admission due to terminal cardiac and/or respiratory arrest, sometimes after an irreversible coma. The children given both Methyl Alcohol and ethyl alcohol survived. At autopsy, one of the children who died had marked edema and softening of the brain.

Aufderheide et al. (1993) reported the cases of two firefighters (aged 22 and 18 years) who were exposed to Methyl Alcohol vapors at the scene of a train derailment. Of the 24 railroad cars that derailed, at least 5 were overturned; 1 contained isobutane, 2 contained Methyl Alcohol, and 2 contained sodium hydroxide. At initial site evaluation, no evidence was found of Methyl Alcohol leak or Methyl Alcohol cloud formation. The two firefighters arrived at the scene, and ran approximately 150 yards from their vehicle between noninvolved railroad cars to reach the hazardous materials (HAZMAT) station. They were wearing short-sleeved shirts, shorts, and shoes without socks. The total estimated time to reach the HAZMAT station was 2 minutes. They did not report any odor at that time, but in retrospect remembered a small cloud developing over the wreckage at a distance of 10 to 20 yards. The air temperature was 90°F, and the air was still. On reaching the HAZMAT station, the men disrobed to their undergarments and donned standard firefighting gear. After 10 minutes of work, both firefighters felt nauseated and dizzy, and one vomited. They and their coworkers interpreted the symptoms to be those of heat exhaustion. Coworkers reported that the men appeared to be confused. The affected firefighters were sequestered from the accident site and transported to a tertiary care facility for evaluation of heat exhaustion and possible chemical exposure.

The 22-year-old reported a mild headache, and was alert and fully oriented. Retinal hyperemia or edema was not detected. The 18-year-old had vague complaints of headache and lightheadedness. He was alert and completely oriented, and the physical examination (including retinal examination) was normal. At approximately 2 hours after exposure, the men had serum Methyl Alcohol concentrations of 19 and 13 mg/dl, respectively. Both firefighters were treated with IV (ethyl) alcohol and folic acid for 48 hours. The peak Methyl Alcohol concentrations were 23 and 16 mg/dl, respectively. The firefighters were discharged after 48 hours with no central nervous system or ophthalmologic sequelae. The initial symptoms were considered due to heat exhaustion, and the serum concentrations of Methyl Alcohol were due to percutaneous and inhalation exposure.

A series of case studies were reported by Wood and Buller (1904). Exposure concentrations and durations of exposure were not reported. A woman used Methyl Alcohol "for weeks" to heat her rheumatic bath and as a facial and head cleanser. The woman had impaired vision (she was unable to distinguish color and form) and partial pallor of the optic nerve. In another case study, a woman used a Methyl Alcohol–fueled lamp to heat water in a poorly ventilated room over a period of 2 to 4 months. The woman had loss of vision that returned to normal after her doctor told her to cease exposure. A cabinet maker had temporary impaired vision after using Columbian spirits (pure Methyl

Alcohol) to remove shellac from his hands. He had headache and vomiting, and his vision was reduced to the perception of light within 10 days from the first symptoms. A man became blind after using Methyl Alcohol to clean his face and arms over a period of 3 years. A man who used alcoholic preparations to dye and clean clothing had failed vision, headache, and vomiting.

Inhalation

Chronic inhalation of Methyl Alcohol vapor caused signs of conjunctivitis, headache, giddiness, insomnia, gastric disturbances, and failure of vision. Workers exposed to average Methyl Alcohol concentrations of 200 ppm for an 8-hour work shift had dimmed vision and nasal irritation, and 3 of 33 workers had unspecified clinical signs of "borderline significance" (Von Burg 1994). An infant that was exposed to a Methyl Alcohol lamp for up to 6 months had contracted pupils, pale optic discs after dilatation, and narrow arteries. The child improved slowly and recovered completely after the parents ceased using the lamp (Wood and Buller 1904).

Methyl Alcohol caused ocular irritation at concentrations of 17,475 ppm in air. Systemic intoxication with Methyl Alcohol typically caused ocular effects related to the onset and severity of metabolic acidosis. These effects included blurred or double vision, constricted visual fields, spots before the eyes, sharply reduced visual acuity, and photophobia or "whiteness." Funduscopic findings were often normal or included peripapillary or retinal edema, and/or hyperemia of the optic disc. Optic atrophy and permanent blindness sometimes resulted from Methyl Alcohol intoxication. Diminution of vision was reported at Methyl Alcohol concentrations of 1200 to 8300 ppm. Chronic effects occurred when Methyl Alcohol exposures exceeded the TLV of 200 ppm; the most significant symptom reported by workers after exposure to >200 ppm was recurrent headache (Von Burg 1994).

Teacher's aides exposed to spirit duplicating fluids containing 99% Methyl Alcohol reported headache, dizziness and/or lightheadedness, blurred vision, and nausea/upset stomach. Twice as many aides (30) qualified for the criterion of Methyl Alcohol intoxication compared to 16 teachers of the control group. The exposures ranged from of 1 h/day for 1 day/week to 8 h/day for 5 days/week over a 3-year period. Ventilation was inadequate or lacking altogether. Exposure concentrations were 475 to 4000 mg/m^3 for 15 minutes initially, and 235 to 1140 mg/m^3 during handling (collating, stapling, etc) of "wet" duplicated papers (Frederick, Schulte, and Apol 1984).

Ocular Toxicity

Methyl Alcohol poisoning causes severe ocular effects in humans, and can result in blindness (Wood and Buller 1904; Browning 1965; Jacobsen and McMartin 1986; Von Burg 1994). Röe (1948) determined that severe acidosis was necessary for the development of amblyopia and amaurosis (blindness). Amaurosis was due to degeneration of the ganglion cells of the retina. In contrast, species of experimental animals such as rats did not have signs of acidosis (see "Animal Toxicology"), and therefore, did not have degenerative changes of the retina.

Röe (1955) reported that the nuclei of ganglion cells were at the extreme periphery of the cells and appeared flattened, polygonal, and irregular, with the nucleolus displaced to the outer edge of the nucleus. Sparse remnants of the tigroid substance were observed arranged in a circle peripherally in the cytoplasm, many of the ganglion cells were enlarged and globoid, and the dendrites were difficult to see.

Skin Irritation and Sensitization

Methyl Alcohol–induced injury to skin occurs by primary irritation, but the danger of blindness and death from inhalation and ingestion are of greater clinical importance (Adams 1983). Prolonged and repeated contact with Methyl Alcohol can result in a defatting action and dermatitis (Kavet and Nauss 1990; Von Burg 1994); however, when metalworkers with dermatitis (94 women and 180 men) were patch-tested with 30% Methyl Alcohol, only 3 women (3.2%) had positive results (Goh and Yuen 1994). Typical allergic responses observed to alcohols are eczematous eruption or wheal and flare at the exposed sites. Eczema and erythema have been reported after the consumption of alcoholic beverages by persons sensitized to ethyl alcohol (Ophaswongse and Maibach 1994).

Methyl Alcohol at a concentration of 5% caused a slight positive (+) reaction in a closed patch test for allergic contact dermatitis (Martin-Scott 1960). In another study, a concentration of 0.7% produced no signs of contact dermatitis, but concentrations of 7% and 70% caused positive (++) reactions (Van Ketel and Tan-Lim 1975). A 62-year-old woman with dermatitis of the lower leg had a (+) eczematous reaction to 10% Methyl Alcohol (48 h exposure). The patient also reacted to other primary alcohols, including ethyl alcohol (1%–100%), and several of their corresponding aldehydes; the woman did not react to 3% formaldehyde (Fregert et al. 1963).

Wilkin and Fortner (1985) reported that humans who were predisposed to ethyl alcohol–induced "flushing" also had reactions to primary alcohols during immediate-type patch-testing. In this study, 25 Orientals with slow-reacting alcohol reductase were challenged with an oral dose of 0.4 mg/kg ethyl alcohol. Cutaneous vascular reactivity was monitored by laser doppler velocimetry. Twelve subjects were then patch-tested with a series of alcohols and aldehydes, including Methyl Alcohol. The ratio of peak to baseline velocimetry values in malar skin after ethyl alcohol challenge was significantly greater in subjects who flushed than in those who did not. Sixty-seven percent of flushers had erythema to topical ethyl alcohol, compared to only one of six nonflushers (control). Methyl Alcohol, however, did not cause erythema under the conditions of this study. Only primary alcohols induced erythema; this reaction was prevented by pretreatment with 4-methylpyrazole. The corresponding aldehydes (e.g., acetaldehyde) provoked erythema that could not be prevented by pretreatment with 4-methylpyrazole. The research

data suggested that the observed cutaneous vascular reaction to primary alcohols was actually provoked by their aldehyde metabolites.

The substrate utilization rates of human cutaneous alcohol dehydrogenase were determined by Wilkin and Stewart (1987) for seven aliphatic primary alcohols, not including Methyl Alcohol. Homogenates of frozen human skin (obtained from grossly normal portion of seven below-knee amputation specimens) were prepared. Alcohol dehydrogenase activity was assayed using the measurement of change in optical density at 340 nm, and enzyme activity was expressed as the amount of NADH generated (nM/mg protein/min) and as relative activity (ethyl alcohol = 1). Enzyme activity ranged from 0 ± 9.6 (2,2-dimethylpropyl alcohol) to 300.9 ± 0.9 nM/mg protein/min (propyl alcohol; relative activity = 3.1).

In the same study, 12 clinically normal subjects participated in an acute patch test. Volumes of 25 μl of ethyl, propyl, butyl, pentyl, 2-methylpropyl, 3-methylbutyl, and 2,2-dimethylpropyl alcohols were pipetted onto filter paper squares that were placed on the volar forearm of each subject. The patches were covered with Parafilm for 5 minutes and then removed. The treated skin sites were observed for erythema at 60 minutes after patch removal. The frequency of erythemogenesis ranged from 16.7% (2-methylpropyl alcohol and 2,2-dimethylpropyl alcohol) to 100% (butyl alcohol, pentyl alcohol, and 3-methylbutyl alcohol). The frequency for ethyl alcohol was 41.7%. The investigators concluded that the frequency of ethythemogenesis correlated strongly and significantly with the rate of substrate utilization by alcohol dehydrogenase (r = .882); the conversion of each primary alcohol to its aldehyde metabolite was therefore correlated with the observed incidence of erythema.

Berardesca et al. (1992) evaluated the potential of 2:1 chloroform-Methyl Alcohol to delipidize the skin and cause erythema. Eleven subjects were treated with 1 ml of the test solution via a glass cup on a skin site on the volar forearm. The exposure time was 1 minute. After removal of the cup, excess solvent was removed using tissue paper. Immediately after delipidization, skin reflectance spectroscopic measurements were performed on treated and untreated skin sites. The measurements were repeated after 1 and 2 hours. Delipidization using chloroform–Methyl Alcohol induced a well-defined erythema. The maximal response was observed 2 to 5 minutes after exposure, and decreased quickly throughout the 2-hour in all but two subjects. Those two subjects had a wheal, which was more pronounced after 1 hour. After 2 hours, erythema was still present, but the wheal had regressed. Immediately after exposure to the test solution, reflectance spectroscopy revealed a 46% increase of total hemoglobin compared to total hemoglobin content in untreated skin. Oxygenized hemoglobin increased by 217% and deoxygenized hemoglobin decreased by 70%. One hour after exposure, oxygenized hemoglobin was increased by 115% and deoxygenized hemoglobin was increased by 9%, compared to control skin. After 2 hours, oxygenized hemoglobin was still increased by 89%, but deoxygenized hemoglobin had normalized. The investigators concluded that the erythema induced by chloroform–Methyl Alcohol was caused primarily by the increase of oxygenized hemoglobin; this increase was likely due to the dilatation of arterioles in the dermal subpapillary plexus, which could have increased the blood flow.

Occupational Exposure Limits

The Occupational Safety and Health Administration (OSHA), the National Institute for Occupational Safety and Health (NIOSH), and the American Conference of Governmental Industrial Hygienists (ACGIH) published exposure limits for Methyl Alcohol. The OSHA permissible exposure limits (PELs) and the NIOSH TLVs are 200 ppm and 260 mg/m^3. The ACGIH TLVs of Methyl Alcohol are 200 ppm and 262 mg/m^3, and bear a "skin" notation, indicating the potential for absorption of toxic amounts through the skin. PEL and TLV values are time-weighted averages for 8-hour work shifts during a standard 40-hour work week. The NIOSH and ACGIH short-term exposure limits of Methyl Alcohol are 250 ppm and 325 to 328 mg/m^3 (NAS 1995; Acros Organics 1996).

In addition to following exposure limit guidelines, workers handling Methyl Alcohol must wear protective eyeglasses or chemical safety goggles, and protective gloves and clothing to prevent skin exposure (Acros Organics 1996).

SUMMARY

Methyl Alcohol is an aliphatic alcohol that is produced by the condensation of hydrogen with carbon dioxide or carbon monoxide at elevated temperature and pressure. It functions as a solvent and denaturant in cosmetic formulations. In data submitted to the FDA in 1998, Methyl Alcohol was reported to be used in four bath preparations. Current concentration of use data are not available, but Methyl Alcohol was used at concentrations up to 5% in 1984.

In humans and animals, Methyl Alcohol was readily absorbed from the gastrointestinal and respiratory tracts and through the skin. The mean rate of absorption through human skin was 0.192 mg/cm^2/min. Methyl Alcohol was absorbed through human cadaver skin such that the peak rate of absorption was reached within 30 minutes of exposure. Only 2% of the dose was absorbed, as most of the applied dose was lost by volatilization. The high water miscibility of Methyl Alcohol allowed the compound to be distributed throughout all organ and tissues in direct relation to the body's water compartment. Methyl Alcohol concentrations in the blood increased during exposure, peaked at or shortly after cessation of exposure, and decreased rapidly thereafter. Regardless of the route of exposure, Methyl Alcohol in the blood was equal to 83% of its aqueous concentration. The concentration of Methyl Alcohol in urine was 20% to 30% greater than in the blood.

Hepatic metabolism in humans accounted for 90% to 95% of the elimination of Methyl Alcohol. In all mammalian species,

the route of metabolism was:

$$\text{Methyl Alcohol} \rightarrow \text{Formaldehyde} \rightarrow \text{Formate } (+H^+) \rightarrow CO_2 + H_2O$$

The formation of formaldehyde was catalyzed by hepatic alcohol dehydrogenase in humans and primates and a hydrogen peroxide/catalase system in other mammals. In Methyl Alcohol–intoxicated animals and humans, formaldehyde did not accumulate substantially in blood, urine, or tissues.

In rats, 14% of a 1 g/kg dose of [^{14}C]Methyl Alcohol was excreted unchanged in the expired air within 48 hours of treatment; the amount of excretion by the kidneys was as great as 10% of the administered dose. In another study >70% of the applied dose (unspecified) was eliminated via the lungs and only a small amount of Methyl Alcohol was oxidized in the tissues; the reverse was true when ethyl alcohol is administered. A review of Methyl Alcohol toxicology reported that unchanged renal excretion of the alcohol accounted for a small amount of elimination after ingestion by humans, and that elimination after overdoses followed saturation kinetics.

In general, only nonhuman primate species presented a model of acute human Methyl Alcohol toxicity. Primates underwent a sequence of early inebriation followed by acidosis, ocular toxicity, formate accumulation, and other signs of toxicity. Other mammals did not undergo the toxic syndrome which was the hallmark of human overexposure. The toxicity of Methyl Alcohol in humans and nonhuman primates was due to the metabolism of the alcohol to formate and formic acid. Formate accumulation caused metabolic acidosis and inhibited cellular respiration.

In rhesus monkeys, four topical applications of Methyl Alcohol treatment (0.5–15 ml, diluted to proportion in antifreeze) caused degeneration of the ganglion cells of the retina, optic nerve, and parenchymal tissues and neurons, as well as fibrosis of the optic nerve. In a 6-day inhalation study, ≥10,000 ppm/day Methyl Alcohol vapors were fatal to all but one rhesus monkey of the 10,000 ppm treatment group. In other studies, treatment of nonhuman primates with Methyl Alcohol or formate resulted in dilated and unresponsive pupils, optic disc hyperemia, edema of the optic disc and retina, and retinal ganglion cell degeneration. Folate-deficient rats, which are unable to efficiently metabolize formate, had signs of retinal toxicity after treatment with Methyl Alcohol. No treatment-related effects were noted when cynomolgus monkeys were treated with up to 6500 mg/m^3 Methyl Alcohol vapors, 6 h/day, 5 days/week for 4 weeks.

Undiluted Methyl Alcohol (0.1–0.5 ml) caused necrosis of the corneal epithelium of rabbits and was a moderate ocular irritant in both in vivo and in vitro studies; using EPA criteria based on the Draize test, Methyl Alcohol was classified as corrosive.

A number of reproductive and developmental toxicity studies have been performed on Methyl Alcohol. In inhalation studies using rats, the maternal NOEL was 10,000 ppm Methyl Alcohol vapors and the NOEL for teratogenic effects was 5000 ppm. When mice were exposed to ≥10,000 ppm Methyl Alcohol vapors during gestation, phase-specific developmental toxicity was observed in the offspring. In whole embryo culture studies (rat and mouse), Methyl Alcohol, formate, and formic acid increased cell death in the forebrain, visceral arches, and the otic and optic placodes.

Methyl Alcohol (23%–31%) had mutagenic effects in the RK$^+$ mutatest, but was not mutagenic in other in vivo or in vitro genotoxicity assays using both microbial and mammalian systems. Carcinogenicity data on Methyl Alcohol were unavailable.

Clinical data show that Methyl Alcohol can cause severe metabolic acidosis, blindness, and death; toxicity was manifested earlier and at a lower dose compared to ethyl alcohol, but the comparative fatal dose was the same for both alcohols. All routes of exposure were toxicologically equivalent, as the alcohol distributed readily and uniformly throughout all tissues and organs. Individual susceptibilities to Methyl Alcohol varied, but typically, the ingestion of 80 to 150 ml of 80% Methyl Alcohol was fatal. Symptoms of Methyl Alcohol intoxication after ingestion were delayed for 12 to 18 hours; afterwards, the symptoms included headache, anorexia, weakness, fatigue, leg cramps, and/or pain and vertigo. Severe gastrointestinal pain, nausea, vomiting, diarrhea, mania, failed vision, and convulsions could occur. Chronic exposure to Methyl Alcohol could cause edema, granular degeneration, and necrosis of heart muscle fibers, as well as fatty degeneration of the heart muscle; sudden cardiac failure was associated with Methyl Alcohol intoxication. The liver and kidneys often had parenchymatous degeneration, and the liver had focal necrosis and fatty infiltration. Severe acidosis was necessary for the development of blindness. Similar symptoms were observed after percutaneous or inhalation exposure to Methyl Alcohol.

In humans, ethyl alcohol had a 10 to 20 times greater affinity for hepatic alcohol dehydrogenase and could decrease the rate of Methyl Alcohol metabolism by approximately 90% via competitive inhibition. Administration of ethyl alcohol also increased urinary excretion of Methyl Alcohol by 1% to 4%. Symptoms of toxicity were milder in patients who consumed both alcohols at the same time and ethyl alcohol could prevent a recurrence of acidosis during alkali treatment.

Methyl Alcohol caused primary irritation to the skin; prolonged and repeated contact with Methyl Alcohol resulted in defatting and dermatitis. In one occupational study, 3.2% of 274 metalworkers with dermatitis had positive results to a patch test of 30% Methyl Alcohol. Typical allergic responses observed after contact with alcohols were eczematous eruption and wheal and flare at the exposure sites. Eczema and erythema were reported after the consumption of alcoholic beverages by persons sensitized to ethyl alcohol. Five percent Methyl Alcohol caused a slight positive (+) reaction in a closed patch test for allergic contact dermatitis, and concentrations of 7% and 70% caused (+++) reactions.

DISCUSSION

The CIR Expert Panel recognized that Methyl Alcohol is toxic via its metabolism to formate. Although current concentration of use data were unavailable for Methyl Alcohol, concentrations of up to 5% are typically used to denature ethyl alcohol in cosmetic products.

Based on the available data, the Panel accepts that Methyl Alcohol will be readily absorbed through the skin, gastrointestinal tract, and respiratory tract and distributed to all organs and tissues in direct relation to the body's water compartment; therefore, all routes of exposure are considered toxicologically equivalent.

The Expert Panel recognized that Methyl Alcohol–induced toxicity was time and concentration dependent. Treatment for Methyl Alcohol poisoning involves administration of ethyl alcohol and hemodialysis, with or without alkali treatment to normalize blood pH. Ethyl alcohol competitively inhibits the rate of metabolism of Methyl Alcohol by up to 90%. Ethyl alcohol also increases the urinary excretion of Methyl Alcohol by 1% to 4%. Symptoms of toxicity were milder in patients who consumed both alcohols simultaneously, and ethyl alcohol treatment prevented recurrences of acidosis. The Panel, therefore, concluded that Methyl Alcohol was safe as used to denature ethyl alcohol for cosmetic products; the Panel has not stated that Methyl Alcohol is safe or unsafe as a solvent.

CONCLUSION

Based on the data presented in this safety assessment, the CIR Expert Panel concludes that Methyl Alcohol is safe as used to denature alcohol used in cosmetic products.

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