NO SIGNIFICANT RISK LEVEL (NSRL)
FOR THE PROPOSITION 65 CARCINOGEN
4-METHYLIMIDAZOLE

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Reproductive and Cancer Hazard Assessment Branch
Office of Environmental Health Hazard Assessment (OEHHA)
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SUMMARY OF FINDINGS

The Proposition 65 "No Significant Risk Level" (NSRL) is defined in regulation as the daily intake level posing a 10^{-5} lifetime risk of cancer. The NSRL for 4-methylimidazole (4-MEI) is calculated to be 29 micrograms per day (µg/day).

The human cancer potency of 4-MEI was estimated and used to calculate the NSRL.[1] The human cancer potency was estimated from dose-response data for lung tumors in male mice exposed to 4-MEI via their feed (National Toxicology Program [NTP], 2007). The potency derivation takes into account body-size differences between humans and experimental animals.

Table 1. Cancer Potency and NSRL for 4-MEI.

Chemical	Cancer Potency $(mg/kg\text{-}day)^{-1}$	NSRL (µg/day)
4-Methylimidazole	0.024	29

INTRODUCTION

This report describes the derivation of a human cancer potency estimate and NSRL for 4-MEI (CAS No. 822-36-6). 4-MEI was listed on January 7, 2011, as a chemical known to the State of California to cause cancer under Proposition 65 (the Safe Drinking Water and Toxic Enforcement Act of 1986; California Health and Safety Code 25249.5 *et seq.*). The listing was based on NTP's findings of clear evidence of carcinogenicity in its studies showing the development of lung cancer in male mice and female mice from 4-MEI exposure (NTP, 2007). Thus, the treatment-related increases in benign and malignant lung tumors in male and female B6C3F$_1$ mice formed the basis of the listing decision. This conclusion was similar to that by the International Agency for Research on Cancer, which found that there was sufficient evidence for the carcinogenicity of 4-MEI in animals (Grosse *et al.*, 2011).

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[1] This document revises the January 2011 OEHHA document describing the derivation of a proposed regulatory NSRL for 4-MEI. The earlier document was part of the Initial Statement of Reasons for the proposed NSRL and was noticed in the California Regulatory Notice Registry on January 7, 2011.

4-MEI is used in the manufacture of pharmaceuticals, photographic chemicals, dyes and pigments, cleaning and agricultural chemicals, and rubber. 4-MEI is formed as a fermentation by–product in ammoniated livestock feed. A main source of 4-MEI exposure to the general public is the presence of 4-MEI in food products resulting from the direct addition of particular types of caramel coloring. Reactions between carbohydrates and ammonia used to produce these caramel colorings form by-products, including 4-MEI (Moon and Shibamoto, 2011). Caramel colorings containing 4-MEI are directly added to some commonly consumed beverages and sauces, including cola, beer, wine coolers, and soy and other sauces (Moon and Shibamoto, 2011; Klejdus *et al.*, 2006; NTP, 2007). In addition, 4-MEI is found in ammoniated molasses and in roasted coffee, but not green coffee, indicating that the compound can also be introduced as a by-product of the roasting process (Casal *et al.*, 2002; NTP, 2007).

The studies available for cancer dose-response assessment and the derivations of the cancer potency estimate and NSRL are discussed below. OEHHA considered all of the data provided in the cited references, as well as the comments provided during the public comment period on the January 2011 proposed NSRL provided by Environ International Corporation (ENVIRON, 2011).[2]

Principles and Assumptions for NSRL Development

Proposition 65 regulations describe default principles and assumptions that apply to quantitative risk assessments used in the establishment of levels posing no significant risk of cancer in the absence of principles or assumptions scientifically more appropriate, codified in Title 27, California Code of Regulations, Section 25703(a).[3] The purpose of the regulation is to specify assumptions and principles for the conduct of risk assessments to produce a No Significant Risk Level which is consistent with the purposes of the Act and which reliably poses no significant risk of cancer.[4] Briefly, these principles and assumptions relate to:

- The quality and suitability of animal bioassay studies and/or epidemiological data
- The use of the most sensitive study
- The application to all routes of exposures for which the results are relevant
- The use of a dose response model that assumes the absence of a carcinogenic threshold dose
- The conversion of rat or mouse cancer potency to human cancer potency
- The use of physiologic, pharmacokinetic and metabolic considerations for inter-species, inter-dose, and inter-route extrapolation
- Human body weights that apply to specific risk subpopulations

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[2] Available at http://www.oehha.ca.gov/prop65/CRNR_notices/index.html.
[3] All referenced sections are from Title 27 of the Cal. Code of Regulations.
[4] Final Statement of Reasons, page 12, Title 27, Cal. Code of Regulations, section 25703 (formerly Title 22, Cal. Code of Regs., section 12703) available at http://www.oehha.ca.gov/ prop65/law/pdf_zip/Art7_8FSRJune1989.pdf.

The default assumptions set forth in the regulation – adopted in 1989 – were based on methods used at the time by state[5] and federal agencies[6] in conducting risk assessments. These methods remain generally accepted and are generally consistent with more recent risk assessment guidance (U.S. EPA, 2005; OEHHA, 2009). There is one exception: the default assumption for conversion of animal to human cancer potency has changed. OEHHA is proposing a change to Section 25703(a) to use this updated and widely accepted approach for the conversion of animal to human cancer potency, and here uses the more recent default in the derivation of the NSRL for 4-MEI.[7]

Additional guidance on the use of mechanistic or mode of action (MOA) information in the assessment of carcinogenic risks from chemicals has been adopted by federal and state agencies (U.S. EPA, 2005; OEHHA, 2009). U.S. EPA's guidance in this area is a valuable resource and is relied upon in the derivation of the NSRL for 4-MEI.

STUDIES AND DATA SUITABLE FOR DOSE-RESPONSE ASSESSMENT

Studies on the Carcinogenicity of 4-MEI

There are no human carcinogenicity studies of 4-MEI. The cancer bioassays that are available and suitable for estimating the potency of 4-MEI were conducted by NTP (2007) in mice.

Proposition 65 regulations specify that "[a]nimal bioassay studies for quantitative risk assessment shall meet generally accepted scientific principles, including the thoroughness of experimental protocol, the degree to which dosing resembles the expected manner of human exposure, the temporal exposure pattern, the duration of study, the purity of test material, the number and size of exposed groups, the route of exposure, and the extent of tumor occurrence" (Title 27, California Code of Regulations, section 25703(a)(1)). The NTP studies in mice meet these criteria. In these studies, NTP (2007) exposed male and female B6C3F$_1$ mice (50 animals/group/sex) to 4-MEI via their diet at concentrations of 0, 312, 625, or 1250 parts per million (ppm) for 106 weeks.

In consideration of tumor occurrence in the NTP rat studies, the NTP concluded that there was equivocal evidence of carcinogenicity in female rats based on the occurrence of mononuclear cell leukemia and no evidence of carcinogenicity in male rats. Section 25703(a)(3) states that "risk analysis shall be based on the most sensitive study deemed to be of sufficient quality." The NTP studies in male and female rats were not sensitive and thus are not used for the dose response assessment. Thus, while the studies were considered, they were rejected for NSRL development due to lack of sensitivity.

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[5] As set forth in California Department of Health Services "Guidelines for Chemical Carcinogen Risk Assessments and their Scientific Rationale," (CDHS, 1985), and California Department of Food and Agriculture, "Risk Assessment Guidelines: Oncogenicity" (March 9, 1987).

[6] U.S. EPA, "Guidelines for Carcinogen Risk Assessment" (1986).

[7] Notice of Proposed Rulemaking, Title 27, Cal. Code of Regs. Proposed Amendment of Section 25703(a)(6), Quantitative Risk Assessment. July 27, 2011.

MacKenzie *et al.* (1992) administered Caramel Color IV (which contains 4-MEI) to mice and rats. However, these studies do not meet the criteria specified in Section 25703(a) because the experiments were not designed to adequately control for and examine the potential carcinogenicity of 4-MEI. In addition, these studies were not of sufficient power to detect an effect of 4-MEI given the group sizes used in the experiment (50 animals per dose group) and the level of 4-MEI present in the test substance. The levels of exposure to 4-MEI in the MacKenzie *et al.* (1992) studies (see Appendix) were 36- to 144-fold lower than the lowest tested dose of 40 milligrams (mg) 4-MEI per kilogram (kg) bodyweight in the NTP studies (see Table 2). Consequently, although the MacKenzie *et al.* (1992) studies were considered, they were rejected for NSRL development due to inadequate study design to evaluate the carcinogenicity of 4-MEI.

Dose-Response Data from the 2007 NTP Mouse Studies

The dose-response data for combined alveolar/bronchiolar adenoma or carcinoma from the NTP studies in male and female mice are presented in Table 2.

Table 2. Incidence of Alveolar/Bronchiolar Tumors in Male and Female B6C3F$_1$ Mice Exposed to 4-MEI via Feed for 106 Weeks (NTP, 2007).

Type of Neoplasm	Concentration in Feed (ppm) [a]				Trend Test [b]
	0	312	625	1250	
Male mice					
Alveolar/Bronchiolar Adenoma or Carcinoma (Combined) [c]	9/49	13/49	16/48	22/49 [d]	p < 0.01
Female mice					
Alveolar/Bronchiolar Adenoma or Carcinoma (Combined) [c]	3/45	8/48	17/48 [d]	14/46 [d]	p < 0.01

[a] Concentrations in feed correspond to calculated average daily doses of 0, 40, 80, and 170 mg/kg-day for both male and female mice, as reported by NTP (2007).
[b] Exact trend test p-values.
[c] The denominator represents the number of mice alive at the time of the appearance of the first alveolar/ bronchiolar adenoma or carcinoma (513 days in male mice and 632 days in female mice).
[d] Significant by pairwise comparison with controls using Fisher's Exact Test (p<0.01).

The NTP administered 4-MEI in feed to male and female mice at the concentrations given in Table 2. NTP estimated average daily dose in mg of 4-MEI per kg bodyweight for each dose group based on the weights of the animals, the amount of chemical added to the feed, and the feed consumption rates. The NTP studies lasted at least 106 weeks and the feed was available every day '*ad libitum.*' NTP estimated average daily doses of 0, 40, 80, and 170 mg/kg-day for mice in these studies. NTP noted that feed consumption was similar overall in dosed groups and control groups. For both male and female mice the incidences of alveolar/bronchiolar tumors were statistically significant using the trend test. For male mice, the incidence of alveolar/bronchiolar tumors was statistically significant in the top dose group. For female mice, the incidences of

alveolar/bronchiolar tumors were statistically significant in the middle and top dose groups.

Survival of dosed groups was similar to that of the corresponding control groups. Mean body weights of male and female mice in the high-dose groups were less than those of controls after 17 weeks and mean body weights of female mice in the low- and mid-dose groups were less than those of controls after weeks 85 and 65, respectively.

EVALUATION OF ALTERNATIVES TO THE NO-THRESHOLD ASSUMPTION

Absent principles or assumptions scientifically more appropriate, Section 25703(a)(5) states that "The absence of a carcinogenic threshold dose shall be assumed and no-threshold models shall be utilized." MOA determination is an approach provided in the 2005 U.S. EPA Guidelines for Carcinogen Risk Assessment for considering if there are scientifically more appropriate principles and assumptions for evaluating the dose-response for a carcinogenic agent. That is the approach relied on here for determining whether an alternative approach to dose-response assessment is more scientifically appropriate than the no threshold approach.

In a MOA determination, a clear explanation is provided of the critical events following exposure to an agent that lead to the development of tumors. U.S. EPA describes a MOA as follows:

> "The term 'mode of action' is defined as a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation. A 'key event' is an empirically observable precursor step that is itself a necessary element of the mode of action or is a biologically based marker for such an element. Mode of action is contrasted with 'mechanism of action,' which implies a more detailed understanding and description of events, often at the molecular level, than is meant by mode of action. The toxicokinetic processes that lead to formation or distribution of the active agent to the target tissue are considered in estimating dose but are not part of the mode of action as the term is used here. There are many examples of possible modes of carcinogenic action, such as mutagenicity, mitogenesis, inhibition of cell death, cytotoxicity with reparative cell proliferation, and immune suppression." (U.S. EPA, 2005; p. 1-10).

MOA analysis includes physical, chemical and biological information and the entire range of information developed in the assessment that contributes to a reasoned judgment concerning the plausibility of potential MOAs (U.S. EPA, 2005). Further, the MOA should be established with reasonable scientific certainty, not simply hypothesized as the "most likely."

U.S. EPA also says:

> "Elucidation of a mode of action for a particular cancer response in animals or humans is a data-rich determination. Significant information should be developed to ensure that a scientifically justifiable mode of action underlies the process leading to cancer at a given site. In the absence of sufficiently, scientifically

justifiable mode of action information, EPA generally takes public health-protective, default positions regarding the interpretation of toxicologic and epidemiologic data: animal tumor findings are judged to be relevant to humans, and cancer risks are assumed to conform with low dose linearity." (U.S. EPA, 2005; p. 1-10)

Evidence Regarding Possible Mode(s) of Action

As noted above, there are numerous possible modes of carcinogenic action. These include various types of genotoxicity, stimulation of cell growth, inhibition of cell death, receptor activation, and events related to the suppression of immune surveillance. Generally, positive experimental evidence in any of a number of types of tests for genotoxic potential is considered to provide evidence that that activity may play a role in the chemical's carcinogenic MOA, absent convincing evidence that a different MOA is exclusively operative. The absence of evidence of genotoxicity does not identify a MOA. Because genotoxic activity has the potential to produce heritable changes to cells that may result in their progression toward malignancy, evidence of genotoxicity is considered to provide support for a non-threshold approach to the dose-response assessment. Non-genotoxic MOAs also have the potential to be linear at low doses, or to have a threshold for carcinogenic action which is low enough that a linear model at environmentally relevant doses is a reasonable approximation of the relationship between exposure and risk (National Research Council, 2009, Chapter 5).

Genotoxicity

4-MEI has been tested for mutagenicity in *Salmonella typhimurium*, *Escherichia coli*, and *Klebsiella pneumonia*. Test conditions and results are presented in Table 3 below. Overall, the *in vitro* bacterial tests provide no evidence for the mutagenicity of 4-MEI.

4-MEI has been evaluated in bone marrow micronucleus tests in rats and mice (NTP, 2007). Male rats and male mice were injected intraperitoneally with 4-MEI at doses of 0, 25, 50, or 100 mg/kg (three injections once a day), and evaluated one day after the last injection. Rats were tested in a single trial; mice in two trials. No increase in micronuclei was observed in the bone marrow cells of treated rats. The first trial in male mice showed a significant increase in micronuclei in bone marrow erythrocytes in the middle and high dose groups; however, a second repeat study did not show significant increases at any dose. It is unclear whether the positive result observed relates to an effect that is important for the development of lung tumors in mice.

4-MEI was also evaluated in a peripheral blood micronucleus test in male and female mice (NTP, 2007). Peripheral blood was examined following treatment of male and female mice with 4-MEI in feed for 14 weeks at doses ranging from 625 to 10,000 mg/kg. No significant increases in micronucleated erythrocytes were observed at any dose.

Table 3. *In Vitro* Bacterial Assays for Mutagenicity of 4-MEI.

Test System	Strain	Results +S9	Results −S9	Activation System	Concentrations Tested	References
***Salmonella typhimurium* (reverse mutations)**	TA97	–	–	Rat or hamster liver S9 (Aroclor 1254-induced)	1 to 10000 µg per plate	NTP, 2007; European Commission, 2000 review
	TA98	–	–			
	TA100	–	–			
	TA1535	–	–			
	TA1537	–	–			
	TA1538	–	–			
***Escherichia coli* (reverse mutations)**	WP2uvrA	–	–	Information not available	9.77 to 5000 µg per plate	European Commission, 2000 review
***Klebsiella pneumonia* (fluctuation test)**	*ur, pro*	NT	–	none	up to 6 mmol/l	Voogd *et al.*, 1979

NT = not tested.

Adequacy of Genotoxicity Testing

4-MEI has tested negative for mutagenicity in multiple experiments in a limited number of *in vitro* bacterial test systems. In *Salmonella* and *E. coli in vitro* experiments, a metabolic activation system was used to increase the chances that a chemical which requires conversion to an active compound for mutagenicity would be detected. However, the metabolic activation system employed was derived from either rat or hamster liver. Since the lung is a primary target tissue in mice, it may be more appropriate to use a metabolic activation system derived from pulmonary tissue. Such test systems have been developed and have resulted in the detection of mutagenic potential for compounds that would not otherwise be detected in traditional liver activation systems (Weems *et al.*, 2010).

The *in vivo* tests examining peripheral blood and bone marrow erythrocytes for micronuclei induction by 4-MEI were generally negative in rats and mice, with the exception of the test in male mice showing an increase in micronuclei in bone marrow erythrocytes. This result was not reproduced in a second trial. Since the lung is a target of carcinogenic activity in mice, the implications of the results in bone marrow and peripheral blood erythrocytes are unclear.

No other tests of genotoxicity for 4-MEI were identified in the available literature, including a number that are commonly used in toxicological evaluations of genotoxic potential: mutations in mammalian cells, mitotic recombination, chromosomal aberrations or sister chromatic exchange in mammalian somatic and germ cells, unscheduled DNA synthesis, gene conversion in yeast or other fungi, DNA strand breaks, DNA damage as measured in the comet assay, 8-hydroxy-2'-deoxyguanosine formation.

Potential for Genotoxic Metabolites

Metabolism of 4-MEI has the potential to influence the carcinogenic response. Knowledge of its metabolism can help inform the approach to the quantitative risk assessment and can provide information on the possible MOA. However, no information regarding metabolites of 4-MEI in humans was identified. Further, little information related to the metabolism of 4-MEI in experimental animals is available, especially in mice, the primary species in which the carcinogenic responses were observed.

NTP reviewed some of the available evidence on the metabolism of 4-MEI (NTP, 2007). Some evidence suggests that 4-MEI is not extensively metabolized in rats exposed by gavage administration, as 85% of the administered dose of 4-MEI was excreted unchanged in the urine of the rats in these studies (Yuan and Burka, 1995). These authors also report a rat urinary metabolite tentatively identified as a sulfate conjugate of 4-MEI. Studies in goats and heifers administered ^{14}C-labelled 4-MEI either intravenously or orally found four metabolites in urine within 24 hours, in addition to the parent compound (Nielsen *et al.*, 1993). One metabolite was unidentified, but the other three were identified as 5-methylhydantoin, 2-methylhydantoic acid, and urea (see Figure 1 below). The amounts of unchanged 4-MEI in urine were ~22% (goats, intravenous administration), ~16% (goats, oral administration), and ~75% (heifers, intravenous administration).

Figure 1. Metabolites of 4-MEI Identified in Goats and Heifers (Nielsen *et al.*, 1993).

4-MEI   5-methylhydantoin   2-methylhydantoic acid   urea
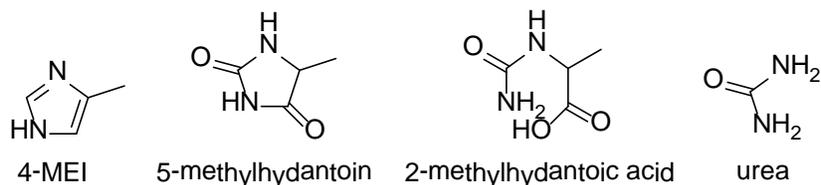
No information on the genotoxicity or carcinogenicity of these metabolites identified from rats, goats, or heifers was found.

NTP also presented a proposed oxidative metabolism scheme for 4-MEI, based on a review of the metabolism of compounds with 5-membered heterocyclic aromatic rings (NTP, 2007; Dalvie *et al.*, 2002; see Figure 2 below, adapted from NTP). Under this proposed scheme, 4-MEI is cleaved following epoxidation of the carbons at the 4/5 position, resulting in the production of methylglyoxal (pyruvaldehyde) and formamidine.

Figure 2. Proposed Metabolic Scheme for 4-MEI (NTP, 2007; Dalvie *et al.*, 2002).

formamidine
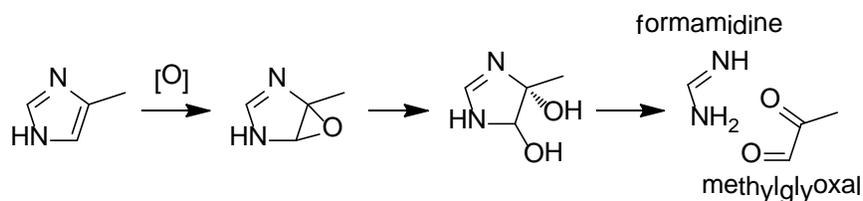
methylglyoxal

As noted above, the data on the metabolism of 4-MEI are scant and this metabolic scheme has not been demonstrated to occur *in vitro* or *in vivo.* At least one putative metabolite, methylglyoxal, has characteristics of concern for potential carcinogenicity.

Methylglyoxal induced injection site fibrosarcomas following subcutaneous administration to Fischer 344 rats (Nagao *et al.*, 1986). It is positive in a number of mutagenicity tests, including *Salmonella* reverse mutation assays, gene *Saccharomyces* conversion assays, and in Chinese hamster lung cells (Nagao *et al.*, 1986; Kasai *et al.*, 1982). Methylglyoxal in drinking water induced glutathione S-transferase placental form-positive foci in the livers of diethylnitrosamine-initiated rats (Hasegawa *et al.*, 1995). Here, the observed increase was significant within the experiment, but was within the range of historical controls. Methylglyoxal also induces oxidative stress and reactive oxygen species in mammalian cells, and induces multiple DNA adducts that are associated with human disease (reviewed by Voulgaridou *et al.*, 2011; citing Kim *et al.*, 2010, Yuan *et al.*, 2008, and Thornalley *et al.*, 2010).

Conclusions Regarding Genotoxicity

Overall, the available literature has provided little evidence for the genotoxicity of 4-MEI. However, the testing has not been adequately comprehensive to rule out a genotoxic MOA, particularly in the lung.

Other Effects of 4-MEI Potentially Related to Its Carcinogenic MOA

Establishing an MOA requires a robust set of data. However, examining even limited available data can help inform what mode(s) of action may be operative for the purpose of generating hypotheses. 4-MEI has undergone testing for various toxicological endpoints, largely for reasons unrelated to its carcinogenicity. The available literature on 4-MEI was examined to determine if there were studies supporting concern for different possible carcinogenic MOAs. Evidence that may be informative from long-term tests that establish carcinogenicity include the nature of the tumor sites and tumor multiplicity, the dose-response in the range of observation, patterns of tumor development over time, the presence of pre-neoplastic lesions, evidence of non-cancer toxicity, latency in tumor development, and the rareness of the tumors (U.S. EPA, 2005; p. 2-37).

Effects on Metabolism

4-MEI has been found to inhibit the metabolism of tolbutamide in male Wistar rats, as indicated by increased half-life and decreased clearance of tolbutamide following intraperitoneal injection (Back and Tjia, 1985). Further work demonstrated the inhibition of tolbutamide hydroxylase in human liver microsomes *in vitro* (Back *et al.*, 1988). Since these studies were conducted, tolbutamide metabolism has been shown to be primarily carried out by the human cytochrome P450 isozyme CYP2C9 (Ohgiya *et al.*, 1992). 4-MEI has also been reported to inhibit the cytochrome P450 isozyme CYP2E1, as measured by the hydroxylation of *p*-nitrophenol in rat liver microsomes (Hargreaves *et al.*, 1994). It is unclear what role, if any, the inhibition of metabolic activity may have on the development of lung tumors by 4-MEI.

Effects on Lung Tissue

Non-cancer lesions of the lung have the potential to shed light on tissue changes in the lung that may have influenced the development of lung tumors in mice. In this section, such changes to the lung observed in NTP's studies are described (NTP, 2007; NTP, 2004).

NTP noted that "[4-MEI] is structurally similar to 2- and 3-methylfuran. Both alkyl furans are metabolized in the Clara cell to reactive species, which may account for their pulmonary toxicity." There are, however, notable differences in the toxicity of these compounds to that of 4-MEI. Inhalation exposure of mice to 3-methylfuran produced necrosis of the Clara cells after one day of exposure (Haschek *et al.*, 1984). Recovery from this lung injury by regeneration was nearly complete within 21 days. A single intraperitoneally-administered dose of 2-methylfuran produced bronchiolar injury to the lung of rats (Ravindranath *et al.*, 1986). Male and female mice exposed once weekly to 3-methylfuran for 10 weeks did not develop lung tumors after two years (Witschi *et al.*, 1985). No long-term studies on the carcinogenic effects of 2-methylfuran were identified. Necrosis of the pulmonary tissue was not observed in the NTP mouse studies of 4-MEI, so the effects observed with these two methylfurans is quite dissimilar.

The lung's Clara cells function in the metabolism of compounds, the regulation of the immune system, and as progenitor cells (Reynolds and Malkinson, 2010). Clara cells have been proposed to be the origin of certain lung tumors, particularly in humans. No information was located suggesting that 4-MEI causes lesions to the Clara cells of mice or any other species.

In conclusion, no specific MOA for tumor development is suggested by the available data on the lung effects of 4-MEI.

Dose-Response in the Observable Range

An inspection of the cancer dose-response data from the studies of 4-MEI in male and female mice shows a pattern consistent with a linear-dose response relationship for both male and female mice, and no evidence for a threshold.

Overall Evaluation of Possible Alternative Approach to the No-Threshold Assumption

As noted above, under Proposition 65 regulations, "[t]he absence of a carcinogenic threshold dose shall be assumed and no-threshold models shall be utilized" (Section 25703(a)(5)), "in the absence of principles or assumptions scientifically more appropriate, based upon the available data" (Section 27703(a)).

The U.S. EPA (2005) provides guidance on when an alternative (*i.e.*, non-linear) approach may be more appropriate:

> "A nonlinear approach should be selected when there are sufficient data to ascertain the mode of action and conclude that it is not linear at low doses and the agent does not demonstrate mutagenic or other activity consistent with linearity at low doses." (U.S. EPA, 2005; p. 3-22)

Presently, there are few data to provide a basis for determining the MOA for 4-MEI's carcinogenicity. Neither the mechanism(s) nor mode(s) of action of carcinogenicity of 4-MEI is known. And data that could be used to hypothesize particular modes of action are extremely limited. Testing has not been sufficiently robust to rule out possible genotoxicity in the target organ. Studies to explore the possible modes of action, let alone studies that would provide a strong basis for assuming a particular MOA, have not been performed. There is not sufficient evidence to justify departing from the default

assumption.[9] In addition, the NTP mice data are consistent with a linear dose response relationship.

Therefore, the default approach using a linearized multistage model, outlined in Section 25703, is applied to derive a cancer potency estimate. This approach is also consistent with recent U.S. EPA (2005) and OEHHA (2009) guidance and practice, as noted above.

EVALUATION OF POSSIBLE PHARMACOKINETIC ADJUSTMENTS

Physiologic, pharmacokinetic and metabolic considerations may be taken into account in quantitative risk assessments under Proposition 65 regulations for inter-species, inter-dose, and inter-route extrapolations, when the data are of such quality that they may be taken into account with confidence (Section 25703(a)(7)).[10]

Evidence for Possible Pharmacokinetic Adjustments

There is limited information available on the absorption, distribution, metabolism, and elimination of 4-MEI. Some data are available from mice (NTP, 2007), rats (Yuan and Burka, 1995), sheep (Karangwa *et al.*, 1990), and goats and heifers (Nielsen *et al.*, 1993). No data from studies in humans were located.

Pharmacokinetic data from mice, the species in which the key carcinogenic findings were reported, are very limited. The NTP conducted single dose studies of 4-MEI in male and female B6C3F$_1$ mice and F344/N rats (NTP, 2007). 4-MEI was administered either by intraperitoneal injection (10 mg/kg body weight) or by gavage (10, 50, or 100 mg/kg) followed by sampling of blood plasma. Absorption half-life estimates were 5 to 23 minutes in rats, and 2 to 5 minutes in mice. Elimination half-life estimates were 65 to 499 minutes in rats and 21 to 87 minutes in mice.

The pharmacokinetics of 4-MEI has been examined more fully in the rat by Yuan and Burka (1995). These investigators treated male F344 rats by either gavage or intravenous administration with radiolabelled 4-MEI. About 85% of a dose of 50 mg/kg administered by gavage was excreted in the urine within two days, with most in the form of 4-MEI, with one minor unidentified metabolite. Other pathways of elimination were minimal (3% fecal; <1% respiration). Dose dependence of elimination suggested that elimination of 4-MEI was saturable. An elimination half-life of ~100 minutes was estimated following an intravenous dose of 5 mg/kg. Formation of a metabolite present in both plasma and urine, tentatively identified as sulfate conjugated 4-MEI, was also found to be a saturable process.

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[9] This is consistent with U.S. EPA's current guidance and practices: "When the weight of evidence evaluation of all available data are insufficient to establish the mode of action for a tumor site and when scientifically plausible based on the available data, linear extrapolation is used as a default approach, because linear extrapolation generally is considered to be a health-protective approach. Nonlinear approaches generally should not be used in cases where the mode of action has not been ascertained." (U.S. EPA, 2005; p. 3-21).

[10] "The toxicokinetic processes that lead to formation or distribution of the active agent to the target tissue are considered in estimating dose but are not part of the mode of action as the term is used here." (U.S. EPA, 2005).

Studies in goats exposed to single doses of radiolabelled 4-MEI by intravenous administration showed that 4-MEI or its metabolites distribute widely to different organs within six hours (Nielsen *et al.*, 1993). A small amount of radioactivity remained one week after administration, suggesting metabolic processes or interaction with cellular systems may occur. Pharmacokinetics from intravenous or oral administration of a single dose were also studied in sheep (Karangwa *et al.*, 1990). These studies showed elimination half-lives of 9 to 10 hours by either route of administration, though plasma levels were highly variable across sheep, particularly following oral administration.

Overall, the results across different experimental animal species suggest relatively rapid absorption and distribution. Elimination half-life estimates were the shortest in mice compared to other species, though the data are quite limited across routes of exposure and doses. No data on the pharmacokinetics of 4-MEI in humans were located.

Conclusions Regarding Pharmacokinetic Adjustments

The absence of information on pharmacokinetics in humans and the scant information available in mice makes confident data-derived pharmacokinetic adjustments to inter-species, inter-dose, or inter-route scaling unfeasible.

CANCER POTENCY DERIVATION

Animal and human cancer potency estimates were derived for 4-MEI by fitting the multistage model (Section 25705(a)(5)) to the dose-response data from the NTP studies in mice. The results are summarized in Table 5 below. Multiplying the animal cancer potency estimate derived from each experiment by the applicable interspecies scaling factor gives an estimate of human cancer potency.

Cancer Potency as Derived from Animal Data

"Multistage" polynomial

For regulatory purposes, the lifetime probability of dying with a tumor (p) induced by an average daily dose (d) is often assumed to be (California Department of Health Services (CDHS, 1985); U.S. EPA, 2002; Anderson and U.S. EPA Carcinogen Assessment Group, 1983):

$$p(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \cdots + q_j d^j)]$$

with constraints, $q_i \geq 0$ for all i. The q_i are parameters of the model, which are taken to be constants and are estimated from the animal cancer bioassay data. With four dose groups, as is the case with the NTP 2007 studies of 4-MEI, the default linearized multistage model has four parameters, q_0, q_1, q_2, and q_3. The parameter q_0 provides the basis for estimating the background lifetime probability of the tumor (*i.e.*, $1 - \exp[-(q_0)]$). The parameter q_1 is, for small doses, the ratio of excess lifetime cancer risk to the average daily dose received. The upper 95% confidence bound on q_1, estimated by maximum likelihood techniques (Crump *et al.*, 1977; Crump, 1984), is referred to here as $q_{1(UCB)}$. When dose is expressed in units of mg/kg-day, the parameters q_1 and $q_{1(UCB)}$ are given in units of $(mg/kg\text{-}day)^{-1}$.

When the experiment duration is at least two years in mice, the parameter $q_{1(UCB)}$ is taken as the animal cancer potency (q_{animal}). When it is shorter than that, an adjustment is applied. Because the NTP (2007) studies of 4-MEI were 106 weeks in duration, a correction factor to extrapolate to 104 weeks was not required and therefore

$$q_{animal} = q_{1(UCB)}.$$

Interspecies scaling

Once a potency value is estimated in animals following the techniques described above, human potency is estimated. According to the current regulation (Section 25703(a)(6)) dose in units of mg per unit surface area is assumed to produce the same degree of effect in different species in the absence of information indicating otherwise. However, a change to that regulation was proposed on July 29, 2011(California Regulatory Notice Register 2011, Volume No. 30-Z), and is in the final stages of adoption. The change is to assume that the amount of chemical per bodyweight scaled to the three-quarters power results in the same degree of effect across species. Under this assumption, scaling to the estimated human potency (q_{human}) is achieved by multiplying the animal potency (*that is,* q_{animal}) by the ratio of human to animal body weights (bw_h/bw_a) raised to the one-fourth power when animal potency is expressed in units $(mg/kg\text{-}day)^{-1}$:

$$q_{human} = q_{animal} \times (bw_h / bw_a)^{1/4}$$

In the 2007 NTP studies, average body weights of 0.0420 kg for male mice and 0.0386 kg for female mice were calculated based on data reported for control animals; the default human body weight is 70 kg. An example derivation of human cancer potency using the male mouse animal cancer potency of 0.00376 $(mg/kg\text{-}day)^{-1}$ is shown below:

$$q_{human} = 0.00376\ (mg/kg\text{-}day)^{-1} \times (70\ kg\ /0.0420\ kg\ )^{1/4} = 0.024\ (mg/kg\text{-}day)^{-1}$$

Cancer potency estimate

Male and female mice showed similar sensitivity to the carcinogenic effects of 4-MEI, with male mice having a slightly higher estimate of human cancer potency. Thus, the human cancer potency estimate of 0.024 $(mg/kg\text{-}day)^{-1}$ was based on the data for male mice.

Table 5. Animal and Human Cancer Potency Estimates for 4-MEI.

Sex, Strain, Species	Type of Neoplasm	Animal Cancer Potency $(mg/kg\text{-}day)^{-1}$	Human Cancer Potency $(mg/kg\text{-}day)^{-1}$
Male B6C3F$_1$ Mice	Alveolar/bronchiolar adenoma or carcinoma (combined)	0.00376	**0.024**
Female B6C3F$_1$ Mice	Alveolar/bronchiolar adenoma or carcinoma (combined)	0.00357	0.023

Bolding *indicates value selected as the basis of the NSRL.*

Cancer risk estimate and risk specific dose

Generally, to estimate risk at low doses, potency is multiplied by lifetime average daily dose in units of mg/kg-day.[11]

$$risk = q_{human} \times dose$$

Therefore, to estimate the dose in mg/kg-day associated with a specific risk, that risk is divided by the cancer potency.

$$risk\ specific\ dose = risk / q_{human}$$

To estimate the risk specific level as an amount per day, the risk specific dose is multiplied by human body weight. The default human body weight for the general population is assumed to be 70 kg (Section 25703(a)(8)).[12]

NO SIGNIFICANT RISK LEVEL

The NSRL for Proposition 65 is a risk specific level of intake associated with a lifetime cancer risk of 10^{-5} (Section 25703(b)). The cancer potency estimate of 0.024 (mg/kg-day)$^{-1}$, based on the combined incidence of alveolar/bronchiolar adenoma or carcinoma in male mice, was used to calculate the NSRL for 4-MEI. A value of 29 μg/day was derived as shown below:

$$NSRL = \frac{10^{-5} \times 70\,kg}{0.024\,(mg/kg\text{-}day)^{-1}} \times 1000\,\mu g / mg = 29\,\mu g / day$$

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[11] The risk estimate obtained is referred to by the U.S. EPA (Anderson and U.S. EPA Carcinogen Assessment Group, 1983; U.S. EPA, 2002) as "extra risk", and is equivalent to that obtained by using the Abbott (1925) correction for background incidence.

[12] When the risk applies to certain subpopulations, the risk specific dose is calculated using different body weights (Section 25703(a)(8); woman (18+ years of age), 58 kg; adolescent (11 – 18 years of age), 40 kg; child (2 – 10 years of age), 20 kg; infant (0 – 2 years of age), 10 kg).

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APPENDIX

Studies published by MacKenzie *et al.* (1992) describe the findings from the long-term administration of Caramel Color IV to F344 rats and B6C3F$_1$ mice. In these studies, 50 animals/sex and each species were provided the test substance in drinking water resulting in dose levels of 0, 0, 2.5, 5 or 10 g Caramel Color IV per kg body weight for 24 months (MacKenzie *et al.*, 1992). No tumorigenic responses were observed in either rats or mice. The test substance was analyzed to contain 110 mg 4-MEI per kg Caramel Color IV. The calculated doses of 4-MEI administered in Caramel Color IV are 0, 0, 0.275, 0.55, and 1.1 mg 4-MEI per kg body weight. Since these studies are not appropriately controlled to examine the potential carcinogenicity of 4-MEI and the doses of 4-MEI were substantially lower than those tested by NTP, OEHHA does not consider the studies to be informative with respect to the carcinogenic dose-response for 4-MEI. NTP's lowest tested dose of 40 mg 4-MEI per kg bodyweight is ~36-fold higher than the highest dose of 4-MEI administered in Caramel Color IV by MacKenzie *et al.* (1992).