FINAL STATEMENT OF REASONS
TITLE 27, CALIFORNIA CODE OF REGULATIONS

SECTION 25705(b). SPECIFIC REGULATORY LEVELS POSING NO SIGNIFICANT RISK

This is the Final Statement of Reasons for a specific regulatory level, namely a No Significant Risk Level (NSRL), for 4-methylimidazole (4-MEI)**,** a chemical listed as known to the State to cause cancer under Proposition 65.[1] On January 7, 2011, the Office of Environmental Health Hazard Assessment (OEHHA) issued a Notice of Proposed Rulemaking for a proposed NSRL for 4-MEI for adoption in Title 27, California Code of Regulations, section 25705(b).[2] The Initial Statement of Reasons set forth the grounds for the proposed amendment. The Initial Statement of Reasons included a technical support document that laid out the scientific basis for the proposed NSRL. A public comment period was provided from January 7 until February 21, 2011. On February 9, 2011, OEHHA extended the public comment period to March 24, 2011, based on a request from Gary M. Roberts of SNR Denton. A public hearing was held on March 10, 2011, to receive comments. OEHHA received two written public comments on March 24, 2011.

On January 7, 2011, OEHHA provided the proposed regulation and initial statement of reasons including the technical support document forming the basis for the proposed regulatory level for 4-MEI to the members of the Carcinogen Identification Committee for their review and comment as required by Section 25302(e). No comments were received from any committee members.

On October 7, 2011, a notice was filed to amend the proposed regulation, modify the technical support document, and add 38 references to the rulemaking file for 4-MEI. A public comment period was provided from October 7 until October 24, 2011. This comment period was extended to November 7, 2011, at the request of Gary M. Roberts of SNR Denton. One comment was received.

The modified proposed regulation and updated technical support document was provided to the Carcinogen Identification Committee members on October 7, 2011. No comments were received from any committee members.

SUMMARY AND RESPONSE TO COMMENTS RECEIVED

Commenters

At the March 10, 2011, public hearing, F. Jay Murray, Ph.D., of Murray and Associates

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[1] The Safe Drinking Water and Toxic Enforcement Act of 1986; Health and Safety Code, section 25249.5 *et seq*.

[2] All further section references are to Title 27 of the California Code of Regulations, unless otherwise noted.

testified but did not object to the proposed NSRL or the regulatory procedure used to adopt the NSRL. Written comments were received during the January 7 - March 24, 2011, public comment period from ENVIRON International Corporation (Principal – Annette M. Shipp, Ph.D.), on behalf of the American Beverage Association and the International Technical Caramel Association, and Chris J. Walker of NOSSAMAN LLP on behalf of the California Small Brewers Association (CSBA).

Additional materials were submitted to OEHHA after the close of the comment period. These were two letters prepared at the request of the American Beverage Association and the International Technical Caramel Association reviewing both the proposed NSRL for 4-MEI and ENVIRON's comments on the proposed NSRL. The letters were from David L. Eaton, Ph.D., Consultant in Toxicology of Mukilteo, Washington (dated July 18, 2011), and Gilbert S. Omenn, M.D., Ph.D., Director, Center for Computational Medicine & Bioinformatics and Professor of Internal Medicine, Human Genetics & Public Health, of the University of Michigan, Ann Arbor (dated July 28, 2011). These materials are included in the record and were reviewed by OEHHA, but are not responded to here because they were received after the public comment period closed.

OEHHA staff also met with a group of individuals representing the American Beverage Association and the International Technical Caramel Association (Annette M. Shipp, Ph.D., Gary M. Roberts, and F. Jay Murray, Ph.D.) on June 6, 2011, after the public comment period closed. The discussion at that meeting did not result in any changes to the proposed NSRL.

After OEHHA's October 7, 2011, revision to the proposed regulation, OEHHA received one comment letter from Michael T. Redman, Vice President, Scientific, Technical and Regulatory Affairs, American Beverage Association, and Dr. Forrest L. Bayer, President, International Technical Caramel Association, dated November 7, 2011. This comment makes reference to the comments previously submitted by ENVIRON.

OEHHA's summary of the relevant comments received during the open comment periods and OEHHA's responses follow. Comments that were not relevant to the adoption of the NSRL for 4-MEI or OEHHA's regulatory procedure are not responded to here.

Response to Comments on Proposed Regulation (Comment period January 7-March 24, 2011)

Public Hearing March 10, 2011

F. Jay Murray, Ph.D., of Murray and Associates testified but did not object to the proposed level or the regulatory procedure used to adopt the NSRL. Dr. Murray asked a series of questions about how the NSRL was developed and calculated. An OEHHA staff member provided answers to these questions. OEHHA did not make any changes to the regulation based on these questions and answers from the hearing.

Written Comments on the Proposed Regulation

Chris J. Walker of NOSSAMAN LLP on behalf of the California Small Brewers Association (CSBA).

No comments relevant to the adoption of the NSRL were made.

ENVIRON International Corporation (Principal – Annette M. Shipp, Ph.D.) on behalf of the American Beverage Association and the International Technical Caramel Association made a number of comments on the proposed regulation, including suggestions on an alternative derivation of the NSRL and criticisms of the scientific methods used to derive the proposed NSRL. The comments were carefully considered. One change to the regulation was made, based in part on these comments. The comments are summarized and responded to below, organized by topic.

Comment 1: <u>Proposition 65 Regulatory Guidance on Quantitative Risk Assessment</u>

The default assumptions on cancer risk assessment guidelines reflected in the Proposition 65 regulations are based on approaches outlined more than 25 years ago by the National Academy of Sciences (NAS 1983) and U.S. EPA (1986). A number of health authorities recommend a weight-of-evidence approach in which all of the data for a chemical are integrated to reach a biologically-based assessment of the potential for a chemical to cause cancer in people (WHO, 2004; RIVM, 2001; U.S. EPA, 2005). This approach has been formally developed by a panel of experts as the Human Relevance Framework (Boobis et al., 2006; Cohen, 2004; Cohen et al., 2003; IPCS, 2005; Meek et al., 2003).

Response:

OEHHA used the Proposition 65 guidance in regulation to derive the NSRL. The guidance sets forth a default approach for deriving NSRLs which are used "in the absence of principles or assumptions scientifically more appropriate" (Section 25703(a)). Thus, the regulations allow the use of more scientifically appropriate approaches for derivation of the NSRL.

As will be discussed below in greater detail in response to other comments, the U.S. Environmental Protection Agency's (U.S. EPA) 2005 Guidelines for Carcinogen Assessment served as a valuable resource in developing the proposed NSRL and in reconsidering the analysis in light of the comments received. The question at hand is whether there is an approach more scientifically appropriate for derivation of the NSRL than the default procedure used by OEHHA. This question turns on whether there is a sufficient understanding of how the chemical causes the cancers seen in the animal studies.

Both the U.S. EPA (2005) Carcinogen Guidelines and the human relevance framework noted in the comments for developing a weight-of-evidence approach to the assessment begin with a consideration of the "mode of action" of the compound. The mode of action is "a sequence of key events and processes, starting with the interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation" (U.S. EPA 2005, p. 1-10). If data are adequate to establish a particular mode(s) of action that is inconsistent with the default approach, under both the U.S. EPA guidelines and the Proposition 65 regulations a non-default approach can be used. Pursuant to Section 25703(a), in order for a non-default approach to be used in the derivation of an NSRL for a particular chemical, the

alternative assumptions and principles must be “more scientifically appropriate” than the default.

With regard to the default assumptions set forth in the Proposition 65 regulation, although these were adopted in 1989, they are generally consistent with more recent risk assessment guidance provided in the U.S. EPA (2005) cancer risk assessment guidelines, and in OEHHA guidance (OEHHA 2009). After the proposed NSRL was released, in part in consideration of the comments received, the default assumption in the Proposition 65 regulation for conversion of animal to human cancer potency was updated. OEHHA amended Section 25703(a) to use a widely accepted approach for the conversion of animal to human cancer potency. The modified proposed NSRL regulation, released October 7, 2011, reflected the proposed Section 25703(a) regulation, which is now finalized and adopted. Therefore, OEHHA used the recently amended default value to derive the NSRL for 4-MEI. The technical support document for this NSRL was also revised to explain in greater detail the application of the regulatory defaults and how the recent amendment to Section 25703(a) was applied to establish an NSRL for 4-MEI.

Comment 2: <u>Human Relevance and Dose-Response for Lung Tumors</u>

ENVIRON’s analysis, and not OEHHA’s, considered a statistical evaluation of the mouse results in combination with other biologically significant data. The mouse tumor responses were not produced by a direct acting genotoxic parent or metabolite. The NTP concluded that the lung tumors in mice arose from Clara cells. Clara cells of the mouse are biologically different and different in number from those of other species, including humans. Therefore, there are more scientifically appropriate data that can be relied on with confidence that the lung tumors are a high dose effect with a threshold that is species-specific. If relevant to humans, the observations are likely to occur by a non-linear, threshold mode of action in humans. An NSRL for 4-MEI is proposed to be at least 20,000 micrograms per day, based on scientifically more appropriate cancer risk assessment principles and assumptions.

Response: OEHHA disagrees with ENVIRON’s conclusions regarding the available data relevant to the carcinogenicity of 4-MEI and their proposed NSRL for 4-MEI of at least 20,000 micrograms per day. Scientific data do not show that “lung tumors are a high dose effect with a threshold that is species-specific.” The MOA for 4-MEI is not known (see response to Comments 3 and 8). It is not known whether genotoxicity is involved in 4-MEI mouse lung carcinogenesis (see response to Comment 4). The key events in mouse lung carcinogenesis have not been identified (see response to Comments 6, 7, and 8). The assumptions embodied in ENVIRON’s threshold/uncertainty factor approach described in the comment are not more scientifically appropriate than those made in determining the NSRL for 4-MEI. The approach OEHHA has used in the calculation of the revised NSRL – a non-threshold assumption and application of interspecies scaling – is consistent with current quantitative assessment practices for carcinogens used by the U.S. EPA.

In adopting an NSRL, OEHHA relies on the guidance on quantitative cancer risk assessment provided in Section 25703. OEHHA has considered the evidence provided by ENVIRON in its comment and finds that the guidance of the authorities identified by

the commenter are not scientifically more appropriate. No changes to the proposed regulation were made based on this comment.

Comment 3: Consideration of <u>Genotoxicity, Pharmacokinetics, and Inflammatory Response in Derivation of the NSRL</u>

The derivation of the NSRL failed to adequately consider the strong possibility that the increase in lung tumors seen in mice was in response to a non-genotoxic, localized mode of action, as evidenced by a lack of mutagenicity or genotoxicity in a battery of in vitro and in vivo tests, the comparative pharmacokinetic data in rats and mice that indicated not only non-linear kinetics but also species difference in which more 4-MEI would be retained by the rat for longer periods of time, and indications of inflammatory responses in the mouse lung, a species with an exceedingly high incidence of spontaneous lung tumors. These data are of sufficient quality that they can be taken into account with confidence.

Response: Each of the factors noted in the comments were considered (See responses to Comments 4-7). As described in OEHHA's modified technical support document, 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 mode of action (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 (U.S. EPA, 2005; National Research Council, 2009)

OEHHA has found that there are presently insufficient 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. 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 (see response to Comment 4). 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. 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). In addition, the NTP mice data are consistent with a linear dose-response relationship.

Physiologic, pharmacokinetic and metabolic considerations may be taken into account in quantitative risk assessments under Section 25703(a)(7) 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. However, in the case of 4-MEI, 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 (see response to Comment 5).

OEHHA does not find the available evidence convincing of a causal role for inflammation or histiocytic infiltration in the development of lung tumors in mice treated with 4-MEI (see response to Comments 6 and 7). For this reason, these findings did not lead to a change in the proposed NSRL for 4-MEI.

With respect to how this comment informs the evaluation of the dose-response relationship, Section 25703(a)(5) states: "The absence of a carcinogenic threshold dose shall be assumed and no-threshold models shall be utilized," absent principles or assumptions scientifically more appropriate. A mode of action 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 OEHHA relied on to determine whether an alternative approach to dose-response assessment (for example, a threshold approach) is more scientifically appropriate than the no threshold approach.

Therefore, the default no threshold approach, outlined in Section 25703, was applied to derive a cancer potency estimate. As explained above, this approach is consistent with recent U.S. EPA and OEHHA guidance and practice. In response to this comment, OEHHA updated its technical support document describing these findings. However, for the reasons described above, OEHHA did not modify its proposed regulation in response to this comment.

Comment 4: <u>Genotoxicity</u>

4-MEI has shown no mutagenicity or genotoxicity in a battery of in vitro and in vivo assays that evaluated the full range of potential for mutagenicity or genotoxicity in prokaryotic and eukaryotic systems, i.e., point mutations, frame shift mutations, or recombination events (NTP 2007). This battery of tests is adequate to determine the lack of mutagenicity and genotoxicity of 4-MEI (U.S. EPA, 2007; ICH, 2008; NTP, 1984), and is of such quality that it can be taken into account with confidence. Although OEHHA referred to the existence of this data, OEHHA did not address the implications of this data for cancer risk assessment. The NTP (2007) concluded from the results of these studies that neither 4-MEI nor its metabolites were shown to be either a direct mutagen or a genotoxicant. The NTP (2007) also stated that "It is unlikely that an alkylating intermediate is involved in mouse lung carcinogenesis in view of the genetic toxicity findings that 4-methylimidazole is not mutagenic in Salmonella typhimurium and does not induce micronuclei in mouse peripheral blood erythrocytes or rat or mouse bone marrow cells." Therefore, 4-MEI is not causing direct genetic changes and such direct genotoxic events could not be the underlying mode of action for the mouse lung tumors seen in the NTP study. Agents that are direct-acting mutagens typically produce

tumors in multiple organs, and often do so in multiple species as well. This is not the case with 4-MEI; a significantly increased response was only noted in the lung of mice and only at the highest doses tested.

Response: The available information on the mutagenicity and genotoxicity of 4-MEI was identified, reviewed and considered in deriving the NSRL regulation. It was described in the January 2011 technical support document and in greater length in the updated October 2011 technical support document. Several *in vitro* and *in vivo* studies in bacteria and rodent species are available. Overall, these available studies provided little evidence for the genotoxicity of 4-MEI. However, as stated in the revised NSRL document, the testing has not been adequately comprehensive to rule out a genotoxic MOA, particularly in the lung, and structural considerations suggest the potential for mutagenic metabolites. These issues are discussed below.

In the experiments in bacteria, 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 lung tissue.

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.

The possibility that genotoxic metabolites of 4-MEI may be generated *in vivo* was also considered in the development of the NSRL. However, no information on the metabolism of 4-MEI in humans was identified and little information on its metabolism in rodents, especially mice, is available. 4-MEI has been proposed to be metabolized to methylglyoxal based on the metabolism of structurally similar compounds. Methylglyoxal is a tumorigenic compound that is also mutagenic and induces DNA damage in experimental systems.

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. Ultimately, 4-MEI has not been adequately tested to rule out genotoxic potential.

While many mutagenic carcinogens produce tumors at multiple sites in rodents, some produce tumors at only one site in one species in standard tests. The finding of tumors at a single site in a single species is not a criterion in U.S. EPA (2005) guidelines for determining whether a chemical acts by a genotoxic mode of action.

Comment 5: Pharmacokinetics

Studies provide evidence that (1) following exposure to higher concentrations of 4-MEI there would be a disproportionate increase in the time it would take for the animal to eliminate 4-MEI (Yuan and Burka, 1995), (2) elimination in rats is mainly of intact 4-MEI and no reactive metabolites were identified (Yuan and Burka, 1995; NTP, 2007), and (3) no accumulation in rats or mice is likely to occur based on absorption and elimination half-lives (NTP, 2007). These results demonstrate differences in kinetics between rats and mice and non-linear, saturable kinetics for the elimination of 4-MEI at the high doses used in the bioassay, and that 4-MEI or any metabolites would be expected to be available for transport and interaction with tissues for longer periods of time in the rat. If 4-MEI were directly responsible for tumor formation, it would be expected that rats should have had greater tumor formation. These data are of such quality that they can be considered with confidence.

Response: Physiologic, pharmacokinetic and metabolic considerations were taken into account in the development of the NSRL, as provided in Proposition 65 regulations. When the data are of such quality that they may be taken into account with confidence, they can be used (Section 25703(a)(7)). Studies described in the comment and studies in other species not identified in the comment were considered by OEHHA in deciding whether to modify the proposed regulation. The pharmacokinetic data are described in the updated technical support document.

OEHHA does not have an explanation for the lack of similar tumor formation in rats compared to mice. Pharmacokinetic considerations may play a role. However, simply because differences in tumor incidence and subtle differences in pharmacokinetic parameters occur between mice and rats does not mean that the pharmacokinetic differences cause the difference in tumor outcomes. Further, since these are comparisons of mice and rats, there are no data that inform how humans may respond.

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. For this reason, OEHHA did not modify the proposed regulation based on this comment.

Comment 6: Inflammatory Responses and Histiocytic Infiltration

Dose-related non-neoplastic effects indicative of inflammatory responses in the mouse lung were seen in both male and female mice in the NTP studies. Significant increases in the incidence of histiocytic infiltration in the lung not only increased with dose but also the severity of the response increased across a dose group. For example, no animal in the control group had this response that was categorized as being in the highest severity category. Only in the highest dose group in male mice was a response categorized as marked, the highest severity level.

Chan et al. (2008) [which summarized the 2007 NTP study results] noted that histiocytic infiltration is often associated with alveolar/bronchiolar carcinomas, although they provided no evidence that infiltration is secondary to tumor development. In the NTP studies, male and female mice exhibited histiocytic infiltration without having tumors. Of the eight male mice with lung carcinomas, seven also had histiocytic infiltration more

severely than control animals. A regression analysis which combined the data for male and female mice showed significant correlation between histiocytic infiltration and lung tumors. When the sexes were considered separately, there was still significant correlation.

Response: The observations described in the comment including histiocytic infiltration were considered in the development of the NSRL and discussed in the revised NSRL technical document. An increase in the incidence of the infiltration of histiocytes (tissue macrophages) in the lung was observed in high-dose female mice in the NTP two-year carcinogenicity studies. A non-significant increase was also observed in high-dose male mice. Chan et al. (2008) characterized this infiltration as "small numbers of histiocytes scattered within alveolar luminae adjacent to many of the adenomas and carcinomas" and stated that histiocytic infiltration and lung tumors are often associated. An IARC scientific publication has noted that in certain lung tumors, the contents of lamellar bodies may be discharged from tumor cells and that infiltrating macrophages may be present to degrade this material.[3] Thus, cellular processes occurring in lung tumors in the mouse are thought to induce the infiltration of macrophages.

Some degree of correlation was observed in the NTP mouse studies between the occurrence of histiocytic infiltration and the development of tumors in individual animals, particularly among high-dose male mice. However, tumors occurred in multiple animals in which histiocytic infiltration was not observed. Histiocytic infiltration was not observed in any of the 18 animals that died before the appearance of the first lung tumors in the two-year studies. Thus, there is no evidence that histiocytic infiltration preceded tumor formation. Since these observations were made at terminal sacrifice (or the time of death for animals that died with lung tumors), the temporal relationship between the two phenomena cannot be established. It is possible that the inflammatory response is secondary to tumor development, as noted in the IARC publication above. The 2004 NTP 14-week studies in male and female mice showed no evidence of treatment-related lung effects of this type. On balance, it is unlikely that histiocytic infiltration plays a role in 4-MEI's carcinogenic MOA in light of the relative weakness of this effect and the commonness of its occurrence as a result of tumor development.

Overall, the available evidence provides no support for a causal role for histiocytic infiltration in the development of lung tumors in mice treated with 4-MEI. These observations cannot be linked to a carcinogenic mode of action for 4-MEI and for this reason, they did not lead to a change in the proposed regulation for 4-MEI.

Comment 7: <u>Inflammation and Clara Cells</u>

NTP (2007) stated that the likely origin of the alveolar/bronchiolar adenomas and carcinomas in mice was the Clara cell in the terminal bronchioles. Consideration of the biology and physiology of the Clara cell in the mouse lung compared to rats and

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[3] Rehm S, Ward JM, Sass B (1994). Tumours of the lungs. In: *Pathology of Tumours in Laboratory Animals. Tumours of the Mouse*. *IARC Scientific Publications*. No. 111. VS Turusov and U Mohr (Eds.). 2nd ed., Vol. 2. Lyon, France: International Agency for Research on Cancer, pp. 325-55.

humans is essential to the evaluation of lung tumors in mice exposed to 4-MEI. ENVIRON noted several specific studies described as showing that (1) inflammation plays a role in mouse lung tumor development, with macrophage infiltration being directly associated with 42% of alveolar/bronchiolar adenomas and with 88% of alveolar/bronchiolar carcinomas (Deng et al., 2010) [ENVIRON noted that this is similar to that observed with 4-MEI], (2) certain viral infections in mice show alveolar adenomatous hyperplasia of the alveolar type II cells and bronchiolar epithelial hyperplasia of the Clara cells (Jackson et al., 2001) [ENVIRON noted that 4-MEI causes lesions similar to epithelial hyperplasia, and this effect has not been identified in humans], (3) many organic compounds injure the Clara cells of the terminal bronchioles in mice and produce tumors and the response for naphthalene is species specific between mice and rats (Plopper et al., 1992), (4) ethylbenzene, styrene, and naphthalene, all non-genotoxic agents, produce lung tumors in mice, but not rats, and this is thought to be due to differences in metabolic capability to produce cytotoxic intermediates (Saghir et al., 2010), (5) mice have more Clara cells in the terminal bronchioles with higher metabolic capacity than rats or humans, making them more sensitive to lung injury (Cruzan et al., 2009).

Response: ENVIRON suggests that the studies identified in the comment provide evidence for a role for Clara cell injury in the carcinogenicity of 4-MEI. This hypothesis was considered when OEHHA developed the NSRL and is discussed in the modified technical document. The lung's Clara cells function in the metabolism of compounds, the regulation of the immune system, and as progenitor cells. Further, Clara cells have been proposed to be the origin of certain lung tumors, particularly in humans. The studies cited in the comment describe experiments showing a potential role for inflammation in the development of some tumor types, and that Clara cells are sometimes affected by certain toxic agents. None of the findings in the comment are shown to be pertinent to 4-MEI's effects in mice. Increases in 4-MEI treatment-related inflammation in the mouse lung were not reported in the long-term or short-term NTP studies. As a note of clarification, *in vivo* styrene is metabolized to genotoxic compounds. There is also evidence for the genotoxicity of naphthalene and ethylbenzene.

While NTP stated that Clara cells were the likely origin of tumors, the NTP did not ascribe this to an inflammation process. No information was found on the potential for 4-MEI to induce inflammation. Chronic focal inflammation was significant in all female rat treatment groups, but without accompanying tumors.

Overall, no information was located suggesting that 4-MEI causes inflammation related to mouse lung carcinogenesis. OEHHA does not find there is compelling evidence that any specific type of lung injury plays a role in the development of tumors by 4-MEI. This issue is discussed in greater detail in the modified technical support document. No changes were made to the proposed regulation based on this comment.

Comment 8: <u>ENVIRON's Proposed Mode of Action</u>

A xenobiotic agent could increase the lung tumor response rate above background in a manner that involves enhanced cell proliferation and decreased apoptosis, leading to the selective proliferation of cells with competent or compromised genetic constructs or

deficient in certain tumor suppressor genes, especially in the Clara cells of the lung.

Response: 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. 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). In addition, the NTP mice data are consistent with a linear dose-response relationship. No changes to the proposed regulation were made based on this comment.

Comment 9: <u>Mononuclear Cell Tumors in Rats and Decreases in Tumor Incidences in Rats</u>

There was a significant increase in the production of mononuclear cell leukemia (MNCL) in female F344 rats in the high dosed group only in the 2007 NTP studies. MNCL is considered to be strain- and species-specific and not relevant to humans. Administration of 4-MEI also produced significant decreases in tumors in several organs in male and female F344 rats, including the adrenal medulla and pituitary gland in male rats and the clitoral gland, mammary gland, pituitary gland, and uterus in female rats.

Response: OEHHA has addressed this subject in the modified NSRL document. Briefly, NTP considers the evidence for each of the four experiments in the 2007 report separately (that is, evidence for male mice, female mice, male rats, and female rats). In consideration of tumor occurrence in the 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. IARC included mononuclear cell leukemia in rats as part of the evidence of carcinogenic effect from animal studies in its review of 4-MEI.

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 the most 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. No changes to the proposed regulation were made based on this comment.

Comment 10: <u>Caramel Color Studies</u>

OEHHA did not consider the MacKenzie et al. (1992) paper in which male and female Fisher 344 rats and B6C3F1 mice were administered caramel color IV containing 4-MEI in drinking water for two years. No significant changes in survival or body weight were

noted after two years of treatment and no increase over controls in either benign or malignant tumors in any organ was found.

Response: The paper of MacKenzie *et al.* was considered in the development of the NSRL. The findings of the 1992 MacKenzie studies are described in the updated technical support document. The publication by MacKenzie *et al.* (1992) describes the findings from the long-term administration of Caramel Color IV to F344 rats and $B6C3F_1$ mice. The studies showed no tumorigenic responses in either rats or mice. However, these studies were not appropriately controlled to examine the potential carcinogenicity of 4-MEI, a contaminant of Caramel Color IV. The doses of 4-MEI were also substantially lower than those tested by NTP, so the studies were not of sufficient power to detect effects in the small numbers of animals used. Therefore, the studies were not informative with respect to the carcinogenic dose-response for 4-MEI. OEHHA did not modify the proposed regulation in response to this comment.

Comment 11: <u>OEHHA's Polygeenan Assessment</u>

OEHHA did not fully consider all of the relevant, scientifically more appropriate data, as WHO (2004), RIVM (2001), and U.S. EPA (2005) would. OEHHA has rarely applied these more scientifically defensible and appropriate approaches. For example, in its assessment of polygeenan (OEHHA, 2001), OEHHA stated that there was strong evidence to suggest that a non-linear mode of action was likely in the production of colorectal tumors in rats. OEHHA stated that there was strong evidence to suggest that production of metaplasia was an important step in the development of the effects in rats and that this induction of metaplasia was "highly non-linear" and, therefore, the "dose-response relationship for polygeenan-induced colorectal tumors likewise would be non-linear". However, because the genotoxicity data were considered by OEHHA to be "insufficient," the default approaches using the linearized multistage model and an outdated interspecies scaling factor were used.

Response: As stated in response to Comment 1 above, the regulatory guidance for risk assessment enables the use of the most appropriate non-default approaches when the scientific data are sufficient to support them. Section 25703 describes default principles and assumptions that apply to quantitative risk assessments when such data are insufficient. While the characterizations of OEHHA's analysis for polygeenan are not correct, its quantitative assessment is not relevant to a determination for 4-MEI, and further discussion of this issue is not needed here. No changes to the proposed regulation were made based on this comment.

Comment 12: <u>Dose-Response in the Observable Range</u>

The increase in lung tumors observed in the mid-dose group of female mice was higher than that noted in the high-dose group in male and female mice in the NTP (2007). This observation is not explained by either survival differences or body weight changes.

Response: OEHHA agrees that the tumor response data in female mice showed a slightly higher incidence in the mid-dose group relative to the high dose group. In evaluating the dose-response data for the purpose of calculating the NSRL, the mathematical model applied takes into account the entirety of the dose-response data, including that of the mid-dose group. Based on the potency determined from the dose-

response data in the experiments conducted in male mice and female mice, the male mice were found to be more sensitive. Section 25703(a)(3) states that "risk analysis shall be based on the most sensitive study deemed to be of sufficient quality." For this reason, the cancer potency derived from male mice served as the basis for the NSRL calculation. No changes to the proposed regulation were made based on this comment.

Comment 13: Selection of the Interspecies Scaling Factor

OEHHA used an outdated species scaling factor based on an assumption that humans are more sensitive than animals according to surface area. Leading public health authorities, such as WHO, RIVM, and EFSA assume that animals and humans are equally sensitive when dose is expressed in milligrams per kilogram per day. Crump et al. (1989), Crump (1989); Allen et al. (1988a,b) and Shipp et al. (1988) provide strong scientific support for this assumption. Another study by Kirman et al. (2003) suggests a scaling factor of body weight to the three-quarters power is recommended when the putative agent is the parent or a stable metabolite, but body weight to the power of one when the putative agent is a reactive metabolite (not necessarily genotoxic). These data are strong enough to depart from the default scaling factor provided in section 25703(a)(6) with confidence. It is unlikely that the parent or a metabolite produced in the liver is the likely active agent in the mouse lung because of differences in pharmacokinetics and responses between mice and rats and the genotoxicity data.

Response: In the case of 4-MEI, the absence of information on pharmacokinetics in humans and the scant information available in mice makes confident data-derived pharmacokinetic adjustments to inter-species scaling unfeasible. Further, since it is unknown whether the agent responsible for the carcinogenicity of 4-MEI is the parent compound or a metabolite (the mode of action is unknown), OEHHA finds that the available data are inadequate to make a determination as to whether metabolic processes may influence the inter-species scaling between mice and humans. For these reasons, OEHHA did not adopt body-weight (power of one) interspecies scaling proposed by the commenter.

The publications from the late 1980s cited in the comment are based on a study of interspecies comparisons of cancer potencies sponsored by the U.S. EPA and other institutions. The U.S. EPA considered the studies' data and analyses in developing its approach to interspecies scaling for estimating human cancer potencies. These analyses provide strong support that for estimating human cancer potency based on animal data. The U.S. EPA (2005) Guidelines for Cancer Risk Assessment considered these and other studies in developing its default approach to interspecies scaling. The U.S. EPA concluded: "The ¾ power is consistent with current science, including empirical data that allow comparison of potencies in humans and animals, and it is also supported by analyses of the allometric variation of key physiological parameters across mammalian species" (U.S. EPA, 2005; p. 3-6).

According to the regulation in Section 25703(a)(6) at the time of the initial proposed NSRL (January 2011), dose in units of milligrams per unit surface area were assumed to produce the same degree of effect in different species in the absence of information indicating otherwise. Since then, that regulation has been amended. Under the amended regulation, the default inter-species scaling assumption is that the amount of

chemical per bodyweight scaled to the three-quarters power will result in the same degree of effect across species. This new interspecies scaling factor was applied in the modified NSRL published on October 7, 2011. This difference resulted in a change in the calculated NSRL from 16 to 29 micrograms per day for 4-MEI. This scaling is the same approach used by U.S. EPA (2005) and other OEHHA programs. Based in part on this comment regarding scaling, OEHHA modified its proposed regulation, resulting in a change in the calculated NSRL.

Comment 14: <u>ENVIRON's Proposed Approach to the NSRL Calculation</u>

ENVIRON conducted an assessment of the toxicity data for 4-MEI that is consistent with the Proposition 65 guidelines. OEHHA should revise its proposed NSRL to at least 20,000 micrograms per day. This assessment used "principles [and] assumptions scientifically more appropriate, based upon the available data" than the default method used by OEHHA. ENVIRON commented that their proposed NSRL is consistent with recent cancer risk assessment guidance of U.S. EPA (2005), WHO (2004), and RIVM (2001).

[Since the dose-response is a threshold phenomenon] a point of departure (POD) should be calculated from the best fitting of the tumor response data in male mice that produced the lowest POD (the LED10 or BMDL10) using a suite of models available from the U.S. EPA (2010). ENVIRON commented that the scientifically appropriate Uncertainty Factors (UFs) should be 10 for human variability and 10 for extrapolation of mouse to humans. Based on the total UF of 100 (10 x 10) and the POD, an NSRL of at least 20,000 micrograms per day is scientifically more appropriate.

Response: OEHHA has carefully considered the alternative approach proposed by ENVIRON in the comments using a calculated point of departure (POD) and the application of uncertainty factors to derive an NSRL. The approach is not consistent with the cancer risk assessment guidelines in Section 25703 or of U.S. EPA. Under U.S. EPA and WHO guidance, the mode of action proposed would have to be strongly supported by data. The mode of action for 4-MEI carcinogenesis is completely unknown. The WHO guidance being cited was draft. WHO's finalized guidance (WHO, 2005) emphasizes the importance of evidence in evaluating mode of action.[4] While ENVIRON supplied a hypothesis for how 4-MEI may cause cancer (with a role for inflammatory responses and selective cell proliferation), ENVIRON has not supported its hypothesis with a robust body of scientific data.

As described in the modified technical document for this NSRL, there are a number of plausible modes of action for the carcinogenesis of 4-MEI, none of which has the level of evidence necessary to depart from the non-threshold assumption specified in the Proposition 65 regulations, and which would be consistent with U.S. EPA (2005) guidance. Based upon OEHHA's interpretation of Section 25703 and consistent with U.S. EPA approaches to the assessment of risks from exposure to carcinogens,

[4] WHO (2007). World Health Organization. IPCS Framework for Analyzing the Relevance of a Cancer Mode of Action for Humans and Case-Studies. Harmonization Project Document No. 4.

OEHHA finds there is not adequate information available to conclude that the tumorigenic response in mice to 4-MEI is a threshold phenomenon.

The principles and assumptions specified in the regulations provide for the approach to interspecies scaling. ENVIRON's proposed uncertainty factors described in the comment are not directly applicable to the non-threshold approach adopted by OEHHA. Here again, OEHHA has considered whether the evidence submitted by ENVIRON would lead to a change in OEHHA's approach to calculating the NSRL for 4-MEI. ENVIRON does not provide sufficient evidence that leads OEHHA to find that a departure from the non-threshold default assumption is scientifically more appropriate. Thus an approach which applies uncertainty factors to a POD was not undertaken. The non-threshold approach used in the calculation of the revised NSRL is consistent with current quantitative assessment practices for carcinogens by U.S. EPA and the Proposition 65 regulations. No change to the proposed regulation was made based on this comment.

Comment 15: <u>WHO Assessment Practices</u>

The World Health Organization (WHO) expresses conclusions about the lack of a significant cancer risk by identifying what it calls an acceptable daily intake (ADI; WHO 2004). A cancer-based ADI is the amount of a chemical to which a person could be exposed daily for a lifetime without encountering unacceptable risk of cancer. As a general rule, for compounds categorized by IARC as a Group 1 or Group 2A carcinogen, ADI values are derived using a non-threshold approach using quantitative risk assessment with low-dose risk extrapolation (WHO 2000). For compounds in Groups 2B, 3, and 4, ADI values are derived with the use of a threshold method. However, if chemicals in Groups 1 and 2A have sufficient evidence of non-genotoxicity, then a threshold method may be applied for carcinogenic risk assessment.

Derivation of the ADI using the threshold method can be achieved using either the NOAEL approach or BMD modeling to derive a BMD and BMDL. WHO uses an UF of 100 to convert the NOAEL from an animal study into a health-based guidance value (WHO 2004). The default 100-fold UF represents the product of two 10-fold factors: interspecies differences and human variability. In addition, chemical-specific adjustment factors (CSAFs) are introduced to allow appropriate data on species differences and/or human variability in toxicokinetics or toxicodynamics to modify the default 10-fold uncertainty factor. The original 100-fold UF can be replaced with CSAFs.

When the genotoxicity data are weak or the response in an animal model are not considered to provide sufficient basis for designation of a chemical as a potential human carcinogen, WHO will use non-cancer toxicity data to develop an ADI. An example is their evaluation of styrene. While the genotoxicity data were weak but of mixed results, the lung tumors in mice were not considered to be representative or predictive of potential human health effects; therefore, WHO used other non-cancer data, evidence of neurotoxicity, and the endpoint upon which to base the ADI for styrene. Application of the WHO approach to the mouse bioassay data resulted in an equivalent NSRL of approximately 28,000 to 20,000.

We conservatively applied the WHO guidance to derive an ADI/NSRL in two ways: 1) based on the highest NOAEL below the lowest LOAEL in either sex, which was 40

mg/kg/day based on data in female mice (an approach more conservative than EFSA's approach, which selected a higher NOAEL of 80 mg/kg/day); and, 2) application of a suite of Benchmark models to data from male or female mice and the selection of the lowest BMDL with the best fit from among the data for male and female mice, which in this case was the male mouse. A BMDL of 29.1 mg/kg/day was selected as the POD.

Response: WHO has not conducted a quantitative cancer risk assessment of 4-MEI in its 2000 Air Quality Guidelines for Europe. Therefore, it is not possible to know whether such an assessment would feature the application of a threshold model. IARC, a part of the WHO, has recently classified 4-MEI as possibly carcinogenic to humans (Group 2B), based on the findings of lung cancer in male and female mice and leukemia in rats.[5]

Procedures and assumptions regarding carcinogens vary across different regulatory and public health agencies, particularly outside the U.S. In 2009, the WHO published guidelines on drinking water quality.[6] In this guidance, WHO advises the evaluation on a case-by-case basis of compounds shown to be carcinogens in order to make distinctions between the underlying mechanisms. The assessment approach is driven by a determination of the mode of action, which includes evidence of "genotoxicity, the range of species affected the relevance to humans of the tumours observed in experimental animals and the toxicokinetics of the substance." A threshold approach is used for carcinogens "for which there is convincing evidence to suggest a non-genotoxic mechanism or to suggest that detoxification mechanisms require to be overwhelmed by high doses."

WHO also notes here that "IARC policy is that its classification should not be used to directly inform regulatory activity. This is because of the importance of taking account of underlying mechanism." This represents advice to not merely use the IARC classification scheme for carcinogens to determine quantitative approaches to assessment.

The approach taken by OEHHA in the determination of the NSRL for 4-MEI is more consistent with that outlined in WHO's drinking water quality guidelines than the early air quality guidelines from WHO. That is, in the WHO drinking water guidelines, departure from default non-threshold assumptions requires a convincing level of evidence regarding the mode of carcinogenic action. OEHHA disagrees with ENVIRON that there is sufficient evidence to conclude that the mode of carcinogenic action of 4-MEI is known and that it has a threshold. For this reason, no change in the regulation was

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[5] Grosse Y, Baan R, Secretan-Lauby B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Islami F, Galichet L, Straif K, on behalf of the WHO International Agency for Research on Cancer Monograph Working Group (2011). Carcinogenicity of chemicals in industrial and consumer products, food contaminants and flavourings, and water chlorination byproducts. *Lancet Oncology* **12**(4):328-9.

[6] WHO (2009). World Health Organization. WHO Guidelines for Drinking-water Quality. Policies and Procedures used in updating the WHO Guidelines for Drinking-water Quality Public Health and the Environment. Geneva. WHO/HSE/WSH/09.05. Available at URL: http://www.who.int/water_sanitation_health/publications/guideline_policy_procedure/en/.

made in response to ENVIRON's hypothetical WHO assessment of the cancer risk from exposure to 4-MEI and the calculation of a hypothetical NSRL.

Comment 16: <u>EFSA and JECFA Assessment Practices</u>

The European Food Safety Authority (EFSA) recently reviewed the data for caramel coloring in general and 4-MEI specifically. In the review, EFSA concluded that expected intakes of 4-MEI are below the current acceptable daily intake (ADI) for 4-MEI in caramel coloring, so the ADI did not need to be lowered. EFSA concluded that 4-MEI was not carcinogenic in the range of human exposures based on the MacKenzie et al. (1992) study. EFSA concluded that the carcinogenic effect of 4-MEI seen in mice in the 2007 NTP study is a threshold effect, based on the lack of genotoxicity, and that alveolar/bronchiolar neoplasms occur spontaneously at a high incidence in B6C3F1 mice. EFSA concluded that the NOAEL for these effects is 80 mg/kg/day from NTP. EFSA's ADI for 4-MEI was not cancer-based. If it were, EFSA would have used WHO guidance and applied an uncertainty factor of 100 (10 for interspecies and 10 for intraspecies extrapolation) to the identified NOAEL. The NSRL derived pursuant to that approach is approximately 56,000 micrograms per day. ENVIRON commented that an assessment by the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA) would also be approximately 56,000 micrograms per day, because they use the same WHO guidance for the development of safe levels.

Response: The EFSA panel concluded that the carcinogenic effect of 4-MEI seen in the mouse studies was thresholded, and that the intermediate dose of 625 mg 4-MEI/kg diet, equivalent to 80 mg 4-MEI/kg bw/day could be considered to be a NOAEL for these effects. However, EFSA did not provide justification for the statement. The ADI that was adopted by EFSA was not based on a cancer endpoint. Thus, it is not possible to know with certainty how such an assessment would be conducted. JECFA does not appear to have conducted a risk assessment subsequent to the release of the NTP data for 4-MEI. JECFA has long operated under a different series of assumptions than are used in the U.S. JECFA has not and does not practice carcinogen risk assessment as performed in the U.S. Moreover, OEHHA rejects the conclusion that the available evidence convincingly demonstrates that a threshold for carcinogenic action exists for 4-MEI and has not made any changes to the proposed regulation based on this comment.

Comment 17: <u>U.S. EPA Assessment Practices</u>

The U.S. EPA's 2005 Guidelines for Carcinogen Risk Assessment recommends the use of genotoxicity and mode of action data in the assessment of potential carcinogens. These guidelines permit the absence of genotoxicity, the absence of mutagenicity, cytotoxicity and pharmacokenitics to be used by a risk assessment expert to establish a chemical-specific nonlinear risk assessment according to a weight of the evidence approach. The Guidelines do not require a precise understanding of the mechanism of action in order to apply a threshold model for cancer risk assessment.

U.S.EPA's guidance assumes that a human equivalent dose was achieved by scaling based on comparative body weights to the ¾ power, in the absence of pharmacokinetic data to define target tissue doses across species. Human equivalent doses would be evaluated using a suite of statistical dose-response models, in the absence of a

preferred biologically-based model. Each of these models would then be used to estimate a point of departure (POD) at a designated estimated level of response.

When the mode of action is thought to be non-linear, the POD is divided by uncertainty factors to provide an estimate of a level considered to be safe. This non-linear approach is the same as that applied to non-neoplastic effects in the derivation of a Reference Dose (RfD) or Reference Concentration (RfC) (U.S. EPA, 1994, 2005).

Application of this approach results in a reference dose equivalent to a NSRL. The UFs applied are intended to account for uncertainties in extrapolating from experimental conditions to the assumed human scenario (i.e., chronic exposure over a lifetime). Historically, UFs are applied as values of 10 in a multiplicative fashion (Dourson and Stara, 1983). Recent U.S. EPA practice, however, also includes the use of a partial UF of $10^{½}$ (3.162) on the assumption that the actual values for the UFs are log-normally distributed. In the assessments, when a single partial UF is applied, the factor of $10^{½}$ is rounded to 3. Use of either body weight to the ¾ power when conducting dose-response modeling for carcinogens is considered to account for kinetic differences by the oral route in animals and humans (U.S. EPA 2005), when chemical specific kinetic data or a pharmacokinetic model are not available. When a compound is considered to be operating by a non-linear mode of action in the animal model, extrapolation from the POD, as stated is accomplished as is that for the derivation of RfDs/RfCs. Because use of the body weight correction is intended to account for kinetic differences based on the assumption that clearance is slower in humans than rodents, a full factor of 10 is not needed for the UF related to species extrapolation.

"In this application of the U.S. EPA (2005) approach to develop a NSRL, body weight ¾ scaling factor was used in lieu of pharmacokinetic data for doses administered by the oral route, the interspecies scaling factor of the default value of 10 was reduced to a factor of 3 under the assumption that the scaling factor applied accounted for the presumed kinetics between the animal model and humans (U.S. EPA, 2010). However, a factor of 10 was still applied to account for difference in variability in a human population. The total uncertainty factors applied to the POD, defined by the dose-response model selected, in this case, a multistage model, was 30. The resulting NSRL is 10,000 ug/day."

Response: The U.S. EPA has not conducted a quantitative cancer risk assessment of 4-MEI. ENVIRON mischaracterizes the U.S. EPA's cancer risk assessment guidelines. OEHHA disagrees with the hypothetical assessment ENVIRON performed according to its interpretation of U.S. EPA's assessment principles. To say that the guidance permits an assessment to consider the absence of genotoxicity is not the same as saying that U.S. EPA recommends this approach. To the contrary, U.S. EPA's guidance recommends that a non-linear approach should be selected when data are available 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. In the case of 4-MEI, the mode of action has not been ascertained. U.S. EPA has also stated that when the available data are not sufficient to establish a mode of action for a tumor site, the default approach should be linear extrapolation (U.S. EPA, 2005; p. 3-21). Therefore, ENVIRON's assertion that a U.S. EPA assessment would be

conducted in a manner as described in the comment is inconsistent with this guidance. OEHHA made no changes to the proposed regulation based on this comment.

Comment 18: <u>RIVM Assessment Practices</u>

The Dutch National Institute for Public Health and the Environment (RIVM) considers lifetime cancer risk when it derives maximum permissible risk levels (MPRs), which are defined as the amount of substance that any human individual can be exposed to daily during a lifetime without significant health risk. The general rule followed by RIVM when developing carcinogenic MPRs is: where the available (i.e. genotoxicity test results and any other relevant data on the mechanism of action) indicate that the observed effects are the result of a direct interaction with DNA (genotoxic mechanism) a non-threshold approach is warranted. In such a case, a quantitative carcinogenic risk assessment is conducted using a linear extrapolation model that provides a point estimate for cancer risk. For chemicals concluded to be non-genotoxic, as is the case for 4-MEI, RIVM uses a threshold approach which assumes there is a range of exposures from zero to some finite value that can be tolerated without adverse effects. The threshold approach involves the identification of a no observed adverse effect level (NOAEL) that is selected from the available toxicity studies, to which uncertainty factors (UFs) are applied.

In addition, an alternative approach to the NOAEL method identified by RIVM is the application of benchmark modeling to estimate a Benchmark Dose (BMD) and a Benchmark Dose Lower Confidence Limit (BMDL). Advantages of this method include the ability to extrapolate outside the experimental dose range, and the model's ability to respond appropriately to sample size and the associated uncertainty. The BMDL which is the 95% statistical lower bound on the estimated BMD is similar to the NOAEL and can be substituted for the NOAEL in calculating the MPR. The MPR is then calculated by dividing either the NOAEL or the BMDL by a total uncertainty factor. Application of the methods used by RIVM to the mouse bioassay data for 4-MEI resulted in safe levels equivalent to a NSRL of approximately 20,000 to 28,000 ug/day using UFs of 10 for interspecies extrapolation and 10 for human variability.

Response: RIVM is a research institute and independent agency of the Dutch Ministry of Health, Welfare and Sport, that provides its advice on health, nutrition, and environmental protection primarily to the Dutch government. RIVM has not assessed the carcinogenicity of 4-MEI. More generally, there are numerous possible modes of carcinogenic action for 4-MEI. 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. The absence of evidence of genotoxicity does not identify a mode of action and as indicated above, the compound has not been robustly tested for genotoxicity (see response to Comment 4). Also, non-genotoxic MOAs have the potential to be linear at low doses. Therefore, OEHHA disagrees with an assumption that a non-genotoxic chemical can only be assessed by a threshold (non-linear) approach. Since the mode of action is unknown for 4-MEI, OEHHA also disagrees with the commenter that a threshold approach to the quantitative assessment of cancer risk is appropriate for 4-MEI. No changes to the proposed regulation were made based on this comment.

Written Comments on the Modified Proposed Regulation published on October 7, 2011

Michael T. Redman, American Beverage Association, and Dr. Forrest L. Bayer, International Technical Caramel Association made reference to comments previously submitted by ENVIRON. No new comments relevant to the modified NSRL were made, therefore, no responses are given here.

ALTERNATIVES DETERMINATION

In accordance with Government Code section 11346.5(a)(7), OEHHA has, throughout the adoption process of this regulation, considered available alternatives to determine whether any alternative would be more effective in carrying out the purpose for which the regulations were proposed, or would be as effective and less burdensome to affected private persons than the proposed action. OEHHA has discussed why it has not adopted alternative approaches to identifying an NSRL for 4-MEI in response to the technical comments submitted during this regulatory process. OEHHA has determined that no alternative considered would be more effective, or as effective and less burdensome to affected persons, than the proposed regulation.

For chemicals listed under the Act as known to cause cancer, the Act exempts discharges to sources of drinking water and exposures of people without provision of a warning if the exposure poses “no significant risk” of cancer (Health and Safety Code, section 25249.10(c)). The Act does not specify numerical levels of exposure that represent no significant risk of cancer.

The purpose of this regulation is to provide a “safe harbor” level for a particular chemical exposure. This regulation establishes the numerical NSRL for one carcinogen, 4-MEI. At or below this level, the Act does not require a warning regarding cancer or prohibit discharges to sources of drinking water based on carcinogenicity concerns associated with 4-MEI. Thus, this level will allow persons subject to the Act to determine whether a given discharge to sources of drinking water or exposure to people involving these chemicals is subject to the warning requirement and discharge prohibition provisions of the Act related to the risk of cancer (Health and Safety Code sections 25249.6).

Although section 25703 describes principles and assumptions for conducting risk assessments to derive safe harbor levels, many businesses subject to the Act do not have the resources to perform these assessments. Yet each business with ten or more employees needs the ability to determine whether its activities or products are subject to the discharge prohibition or warning requirements of the Act. Given the wide use and occurrence of the chemical covered by this regulation, the absence of this regulation would leave numerous businesses without an efficient way of determining if they are in compliance with the Act without the expenditure of significant resources on their part.

LOCAL MANDATE DETERMINATION

OEHHA has determined this regulatory action will not pose a mandate on local agencies or school districts nor does it require reimbursement by the State pursuant to

Part 7 (commencing with section 17500) of Division 4 of the Government Code. OEHHA has also determined that no nondiscretionary costs or savings to local agencies or school districts will result from this regulatory action. Proposition 65 provides an express exemption from the warning requirement and discharge prohibition for all state and local agencies. Thus, these regulations do not impose any mandate on local agencies or school districts.