On March 15, 2013, the Office of Environmental Health Hazard Assessment (OEHHA) issued a Request for Relevant Information concerning the possible addition of methyl isobutyl ketone to the Proposition 65\(^1\) list of chemicals known to cause reproductive toxicity. The consideration of methyl isobutyl ketone (MIBK) for listing is based on the authoritative bodies provision\(^2\) of the Proposition 65 implementing regulations and the U.S. Environmental Protection Agency’s (U.S. EPA) identification of MIBK as causing reproductive toxicity. This document responds to comments received in response to the Request for Relevant Information.

The U.S. EPA concluded that MIBK causes reproductive toxicity in the 2003 Integrated Risk Information System (IRIS) entry for methyl isobutyl ketone\(^3\) and in its 2003 document, “Toxicological Review of Methyl Isobutyl Ketone; In Support of Summary Information on the Integrated Risk Information System (IRIS)”\(^4\).

U.S. EPA\(^5\) concluded:

“The developmental effects in rats and mice after gestational inhalation exposure are considered to be the most clearly adverse effects in the animal database.”

(page 42)

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\(^1\) The Safe Drinking Water and Toxic Enforcement Act of 1986 (codified at Health and Safety Code section 25249.5 \textit{et seq.}) hereinafter referred to as Proposition 65 or the Act.

\(^2\) Title 27, Cal. Code of Regulations. section 25306.


“… delayed ossification in rats and mice and reduced fetal body weight and increased fetal death in mice were identified as the critical effects in a substantial database of repeat-dose inhalation studies.” (page 36)

“An RfC of 3 mg/m³ was derived on the basis of effects observed in fetuses after repeated exposure on gestation days 6 to 15 (Tyl et al., 1987). The RfC was based on developmental effects in fetuses reported in a toxicity assay in which maternal exposure occurred only during gestation.” (page 41)

U.S. EPA developed an inhalation reference concentration (RfC) based on developmental toxicity manifested as reduced fetal body weight, skeletal variations, and increased fetal death in mice, and skeletal variations in rats6.

Comments responsive to the Request for Relevant Information were submitted by the Ketones Panel of the American Chemistry Council (ACC).

The comments are grouped and numbered by topic, and responses follow below.

1. **Sufficiency of Evidence Criteria for “as causing reproductive toxicity”**

Comment:
The ACC comments reference the U.S EPA test guidelines for developmental toxicity studies and state that MIBK effects are only noted at doses that are higher than those recommended in the U.S. EPA test guidelines for developmental toxicity studies. The ACC noted that:

- “Effects were only noted in the study by Tyl et al. at a dose level that exceeds USEPA’s own guidance for conducting developmental toxicity studies. The IRIS assessment also indicates that maternal toxicity in the study exceeded the levels specified in USEPA’s test guidance.”
- “According to the USEPA guidelines, the highest dose should be chosen to induce some maternal toxicity but not ‘death or severe suffering.’ The guidelines elaborate further that maternal mortality ‘should not be more than approximately 10 percent.’ USEPA’s developmental toxicity guidelines also indicate that the highest dose ‘need not exceed . . . 2 mg/L by inhalation, unless potential human exposure data indicate the need for higher doses.’ The highest dose employed in the study by Tyl et al. (1987) clearly exceeds both of the provisions of the USEPA guidelines.”

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• “The highest tested MIBK dose concentration (12292 mg/m3) in the teratogenicity study was excessively high and should not be considered as a basis for Proposition 65 regulation.”

The comment concludes by stating that:

• “USEPA’s test guidance for developmental toxicity studies clearly represent scientifically valid data, as specified in Section 25306(h) of the Health and Safety Code. The Agency’s failure to reference this guidance in the IRIS assessment, and the assessment’s failure to address the highest-dose criteria outlined in the guidance, provides clear evidence that the 1998 guidance were not considered in the IRIS assessment. As a consequence, USEPA’s 2003 assessment does not satisfy the criteria of ‘as causing reproductive toxicity’ and should not be used as a basis for consideration of listing of MIBK.”

Response:

The U.S. EPA 1998 Health Effects Test Guidelines for Prenatal Developmental Toxicity are explicitly intended to “meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, et seq.), as amended by the Food Quality Protection Act (FQPA)(Pub. L.104–170), and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601)”7. There is no U.S. EPA requirement that studies considered for hazard identification and dose-response assessment under FIFRA or other Agency programs (e.g., Integrated Risk Information System (IRIS)) meet the U.S. EPA Health Effects Test Guidelines testing requirements. Studies considered under the authoritative bodies listing provision of Proposition 65 also are not required to conform to those specific testing guidelines, but instead are evaluated individually relative to the criteria provided in the regulations (Section 25306(g)).

As a point of clarification, the U.S. EPA’s Health Effects Test Guidelines for prenatal developmental toxicity place no limit on doses to be used but instead indicate a highest exposure level that is expected to be adequate under the statutes to which the guidelines are explicitly targeted (i.e., FIFRA, FQPA, and TSCA). As noted by the commenter, these toxicity testing guidelines expressly allow for exposure at a level greater than 2 mg/L [2,000 mg/m^3], indicating that data obtained at such higher dose levels can be relevant to hazard identification.

The commenter’s assertion that the 1998 U.S. EPA Health Effects Test Guidelines constitute scientifically valid data not considered by the authoritative body is unfounded. The test guidelines provide no data whatsoever on the toxicity of MIBK. Moreover, as discussed above, the test guidelines provide no basis for discounting the validity of the study by Tyl et al. (1987) for purposes of risk assessment on the basis of doses used in the study.

**Comment:**
ACC commented on the degree of maternal toxicity in the Tyl et al. (1987) study:

- “The IRIS assessment also indicates that maternal toxicity in the study exceeded the levels specified in USEPA’s test guidance.”
- “The guidelines elaborate further that maternal mortality ‘should not be more than approximately 10 percent.’….As indicated, 3 of the 36 mice dams (12%) [sic] in the study died…”
- “The observed fetal toxicity is indicative of a developmental delay secondary to maternal toxicity and is consistent with external publications (Carney and Kimmel, 2007; Collins et al., 1987; Marr et al., 1992).”

**Response:**
OEHHA assumes that “3 out 36 mice (12%)” expressed in the ACC comment is a typographical error. The published paper by Tyl et al. (1987) reported that 3 of 25 pregnant mice died after the first day of exposure to MIBK, which represents 12% of those animals. However, the paper also reports that 30 mice were included in the experimental group and exposed to MIBK; therefore, the incidence of death in the treated female mice at the high dose was in fact 10% (3 of 30). The paper also reports that “a small number of [animals] (selected randomly) were removed from study at sacrifice since they were not needed to fulfill the Sponsor’s requirement of 25 pregnant animals in each group”. These animals all survived treatment, but subtraction of them from the nominal group size resulted in the smaller denominator used to calculate a maternal mortality incidence of 12%. Thus the degree of maternal mortality in the Tyl et al. (1987) study appears consistent with the upper limit recommended in the U.S. EPA (1998) Health Effects Test Guidelines for Prenatal Developmental Toxicity. It should also be noted that even if the group size was considered to be 25, an exact incidence of 10% maternal mortality would require the death of 2.5 animals. The death of 3 animals resulting in an incidence of 12% maternal mortality can therefore be considered consistent with the guidance that maternal mortality “should not be more than approximately 10 percent.”
The manifestations of fetal toxicity relied upon by the U.S EPA are “reduced fetal body weight, skeletal variations, and increased fetal death in mice, and skeletal variations in rats”. Regarding the relationship between maternal and fetal toxicity, ACC states that its conclusion that observed fetal toxicity is indicative of a developmental delay secondary to maternal toxicity is supported by external publications, but did not provide complete citations for those publications. OEHHA has identified relevant publications consistent with the partial citations provided by ACC, and notes the following statements in those publications:

“Ethylene glycol-induced difference in fetal/pup body weight was apparently the basis for the difference in ossification patterns in EG-treated animals on gd 18 and 20. … Our interpretation of these results is that the effect of ethylene glycol on skeletal ossification is mediated through effects on fetal growth and development as measured by body weight in the prenatal period, gd 18 and 20.” (Marr et al., 1992)

“Decreased ossification of the sternebrae was seen despite the fact that at 27 and 51 mg/kg/day there was no evidence of maternal weight loss or any other toxic effect.” (Collins et al., 1987)

The review by Carney and Kimmel (2007) addresses the general relationship between maternal toxicity and delayed ossification and wavy ribs, but does not address fetal weight or survival. These publications do not provide a basis for concluding that U.S. EPA erred in its interpretation of the relationship between the manifestation of developmental toxicity on which it based its formal identification of MIBK as causing reproductive toxicity and the degree of maternal toxicity reported.

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Comment:
The ACC comment briefly summarized a study by Nemec (2000) and commented that:

- “Nemec reported no reproductive or neonatal developmental effects in either generation of rats exposed to air concentrations of MIBK up to 8219 mg/m³.”

Response:
The study by Nemec (2000) was considered by U.S. EPA, which identified it as WIL Research Laboratories (2000) and discussed it in both of the documents that serve as the basis for formal identification of MIBK as causing reproductive toxicity⁹. As expressed in the IRIS entry for MIBK: “The Tyl et al. (1987) developmental toxicity study was identified as the principal study and is described below; the WIL Research Laboratories (2000) reproductive toxicity study is presented to support the principal study.”

Comment:
The ACC commented that:

- “The IRIS assessment briefly discusses the deaths among the mouse dams [in the Tyl et al 1987 study], in response to public comments, but fails to address the low weight gain in the rat study. The assessment also fails to discuss the appropriateness of the highest dose used in the studies, which is more than 6 times greater than the highest dose level recommended in USEPA guidance (2 mg/L) and well above potential human exposures.”

Response:
The U.S. EPA Toxicological Review¹⁰ supporting the IRIS evaluation states that “Maternal mean body weight, weight gain, and food consumption were significantly decreased in rats exposed to 12,292 mg/m³ (but not to 4106 mg/m³ or lower) during the

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exposure period, but they had recovered to control levels by the day of sacrifice.” Thus, these factors were considered by the authoritative body in the process leading to formal identification of MIBK as causing reproductive toxicity.

The issue of the appropriateness of the highest dose used in the studies has been addressed above in response to another comment. In summary, the U.S. EPA (1998) Health Effects Test Guidelines for Prenatal Developmental Toxicity expressly allow for exposure at a level greater than 2 mg/L by inhalation, indicating that data obtained at such higher dose levels can be relevant to hazard identification.

2. Applicability of U.S. EPA Guidance Documents

Comment:
Regarding U.S. EPA guidance documents, the comments note that:

- “The test guidance [1998 Health Effects Test Guidelines for Prenatal Developmental Toxicity] was in place when the IRIS assessment was conducted, however, and it is not clear why the guidance is not referenced. Instead the IRIS assessment cites 1991 guidance for developmental toxicity risk assessment [1991 U.S. EPA Guidelines for Developmental Toxicity Risk Assessment].”
- This 1991 guidance suggests that excessive toxicity among the dams should not affect the dose-response methodology, but indicates that ‘when maternal toxicity is significantly greater than the minimal maternally toxic dose, developmental effects at that dose may be difficult to interpret.’ The 2003 IRIS assessment makes little attempt to explain this interpretation.”

Response:
The 1991 U.S EPA Guidelines for Developmental Toxicity Risk Assessment provide direction on how data, including toxicological data from experimental studies, are to be interpreted in conducting a risk assessment. In contrast, the 1998 guidelines provide direction on how to conduct a developmental toxicity experiment for submission to meet a regulatory requirement (e.g. for pesticide registration). IRIS risk assessments utilize existing experimental data and so follow the US EPA’s 1991 Developmental Toxicity Risk Assessment guidelines (rather than guidelines on how to test chemicals).

The 1991 guidelines are explicitly intended to be applicable to the interpretation of data not only from studies that follow regulatory testing guidelines but also from studies that follow other testing protocols, as noted in the Guidelines themselves:
These Guidelines provide a general format for analyzing and organizing the available data for conducting risk assessments. The Agency previously has issued testing guidelines (U.S. EPA, 1982b, 1985a, 1989a, 1991a) that provide protocols designed to determine the potential of a test substance to induce structural and/or other adverse effects during development. These risk assessment Guidelines do not change any prescribed statutory or regulatory standards for the type of data necessary for regulatory action, but rather provide guidance for the interpretation of studies that follow the testing guidelines and, in addition, provide limited information for interpretation of other studies (e.g., epidemiologic data, functional developmental toxicity studies, and short-term tests) that are not routinely required, but may be encountered when reviewing data on particular agents.

Thus, the following statement from the U.S. EPA Toxicological Review of Methyl Isobutyl Ketone is entirely consistent with U.S. EPA’s stated purpose for its 1991 Guidelines for Developmental Toxicity Risk Assessment:

“Development of these hazard identification and dose-response assessments for methyl isobutyl ketone has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: Guidelines for the Health Risk Assessment of Chemical Mixtures (U.S. EPA, 1986a), Guidelines for Mutagenicity Risk Assessment (U.S. EPA, 1986b), Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991a)....”

As noted above, U.S. EPA IRIS assessments are not limited to consideration only of studies conducted for purposes of pesticide registration under FIFRA or those that used the 1998 testing guidelines.