October 7, 2013

Ms. Cynthia Oshita
Office of Environmental Health Hazard Assessment
State of California
1001 I Street
Sacramento, CA 95814

Re: Notice of Intent to list methyl isobutyl ketone as known to the State to cause reproductive toxicity under the Safe Drinking Water and Toxic Enforcement Act of 1986, September 6, 2013

Dear Ms. Oshita:

The Ketones Panel of the American Chemistry Council submits the following information in response to the Office of Environmental Health Hazard Assessment's (OEHHA) notice of intent to list methyl isobutyl ketone (MIBK) as a reproductive toxicant under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65). The Ketones Panel represents US manufacturers of MIBK and other ketones.

The September 6, 2013 notice indicates that listing of MIBK is being considered under the authoritative bodies mechanism (Health and Safety Code § 25306), based on the 2003 update of the US Environmental Protection Agency's (USEPA) Integrated Risk Information System (IRIS) assessment for MIBK.[1] As part of this update, USEPA calculated a reference dose (RfD) for developmental effects in rats and mice exposed to a massive dose of MIBK in a study by Tyl *et al.,* 1987.[2]

HSC § 25306(h) specifies that a chemical does not satisfy the definition as a reproductive toxicant "if scientifically valid data which were not considered by the authoritative body clearly establish that the chemical does not satisfy the criteria" for listing under Proposition 65. These listing criteria include –

- studies in humans indicating a causal relationship between the chemical and reproductive toxicity, or

[1] USEPA. Toxicological Review of Methyl Isobutyl Ketone: In Support of Summary Information on the Integrated Risk Information System (IRIS). EPA/635/R-03/002 (March 2003).

[2] Tyl et al. Developmental toxicity evaluation of inhaled methyl isobutyl ketone in Fischer 344 rats and CD-1 mice. *Fund Appl Toxicol* 8:310-327 (1987).

- studies in experimental animals indicating that an association between adverse reproductive effects in humans and the chemical is biological plausible.[3]

Appendix A of the IRIS assessment for MIBK indicates that USEPA considered "the totality of effects at the highest concentration as co-critical (delays in ossification, decreases in fetal body weight, and increased fetal death)" yet provides no explanation how much weight was given to each of the specific end points.[4] As a consequence, one must assume that the IRIS document gives equal weight to each of the three endpoints

The Ketones Panel submitted the enclosed comments in response to the Office's March 2013 call for information. These comments noted that the effects used as the basis for the RfD in the IRIS document occurred at an excessively high dose that caused levels of maternal toxicity that make the results difficult to interpret. In response, OEHHA suggests that the concerns raised by the Panel were considered by USEPA as part of the IRIS assessment of MIBK. For the reasons outlined below, the 2003 IRIS assessment does not provide sufficient basis for the Proposition 65 listing since it failed to consider relevant and scientifically valid data that were available at the time or that have since become available challenging USEPA's conclusion.

USEPA Failed to Consider the Researchers' Conclusion that the Reductions in Fetal Body Weight Was Not Treatment-Related

Fetal body weights were significantly reduced in the offspring of rat dams exposed to the lowest and highest doses in the Tyl *et al.* study, but not in the middle dose. In mice, fetal body weights were reduced in the highest dose group, but not in either of the lower exposure groups. Statistical analysis conducted as part by the study by Tyl *et al.* indicates that the reduction in the rat fetal body weights was confounded by a skewed distribution of litter size. Based on their analysis, the authors concluded that the reductions in rat fetal body weight were not treatment related. Among the offspring of the mouse dams, a reduction in fetal body weight only occurred at the highest dose for which the authors observed clear evidence of significant maternal toxicity (*i.e.*, increase in deaths, clinical signs, and increased absolute and relative liver weight).

The IRIS assessment provides no analysis of the fetal body weight data reported by Tyl *et al.*, other than to report the findings of the study. Consequently, it is not clear what consideration USEPA gave to the conclusions of the study authors regarding the significance of the body weight data, if any.

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[3] HSC § 25306(g).

[4] USEPA 2003, at 57. The IRIS document further indicates that EPA used a "constellation" of developmental effects at the highest dose from the Tyl *et al.* study. EPA provides no other information on other effects that were considered beyond the three previously identified.

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USEPA's Assessment Failed to Consider OECD Guidance for Prenatal Developmental Toxicity Studies Adopted in 2001

The Organization for Economic Co-operation and Development (OECD) updated its guideline for the design of prenatal developmental toxicity studies[5] in 2001 which, according to the documentation, was proposed by USEPA. As part of this update, the OECD provides the following guidance for dosing limits –

> If a test at one dose level of at least 1000 mg/kg body weight/day of oral administration . . . produces no observable toxicity and if an effect would not be expected based upon existing data (e.g., from structurally and/or metabolically related compounds), then a full study using three dose levels may not be considered necessary. Expected human exposure may indicate the need for a higher oral dose level to be used in the limit test. For other types of administration, such as inhalation or dermal application, the physical chemical properties of the test substance often may indicate the maximum attainable level of exposure.

The OECD guideline further explains that –

> [t]he results of the study should be interpreted in conjunction with the findings of subchronic, reproduction, toxicokinetic, and other studies. Since emphasis is placed on both general toxicity and the developmental toxicity endpoints, the results of the study will allow for the discrimination between developmental effects occurring in the absence of general toxicity and those which are only expressed at levels that are also toxic to the maternal animal.[6]

The IRIS documentation makes no reference to the OECD guideline, despite the fact that USEPA was actively involved in its implementation. USEPA does not discuss the guidance related to doses above 1000 m/kg body weight – half that of USEPA's previous 1991 guidance[7] and more than an order of magnitude below the top dose used by Tyl *et al.* which resulted in the "constellation" of effects considered by USEPA. Nor does the IRIS document consider the findings of Tyl *et al.* in conjunction with the other available information, as indicated by OECD. USEPA makes no attempt to discriminate between effects occurring in the absence of general

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[5] OECD. Guideline for the Testing of Chemicals. Guideline 414 – Prenatal Developmental Toxicity Study. Adopted 22 January 2001. Available at http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788;jsessionid=bb72c4wtiijq.delta.

[6] Id., at 8/11.

[7] USEPA. Guidelines for Developmental Toxicity Risk Assessment. Risk Assessment Forum. EPA/600/FR-91/001 (December 1991). Available at http://www.epa.gov/raf/publications/pdfs/DEVTOX.PDF.

toxicity and those occurring at doses resulting in maternal toxicity, as directed in the EPA-recommended revisions to the OECD guideline.

USEPA Failed to Consider Whether Potential Human Exposure Data Indicate the Need to Test Exposures Above 2 mg/L, as Outlined in Its 1991 Guidelines

In interpreting effects observed at doses that exceed both USEPA's 1991 guidance and the updated 2001 OECD guidance, consideration must be given to whether expected human exposures indicate the need for a higher dose level. In its response to the Panel's March 2013 comment, OEHHA notes that the USEPA guidelines "expressly allow for exposure at a level greater than 2 mg/l [2000 mg/m3]" and as a result "such higher dose levels can be relevant to hazard identification."

Neither OEHHA nor USEPA provide any evidence to suggest that the excessively high exposures used in the study by Tyl *et al.* are relevant to human exposures. Clearly, the study would not have included such high doses had it been conducted after the 1991 guidelines were in place. Interpretation of these data demands a consideration of their relevance to hazard assessment – particularly in light of other data that exist. However, no such consideration is provided in the IRIS documentation.

USEPA's Assessment of MIBK Failed to Consider the Results of the Two-Generation Reproduction Study in Assessing the Potential for Developmental Effects

OECD guidance stresses the importance of considering results from reproduction and other relevant studies in interpreting developmental toxicity study results. The two-generation reproduction study by Nemec (2000) provides a robust data set for MIBK both in terms of sample size and evaluated endpoints.[8] Its sound scientific quality make it a relevant study from which to assess potential developmental effects – particularly in light of the excessively high doses used in the study by Tyl *et al.* USEPA's IRIS assessment does not include such consideration.

More Recent Interpretation of Skeletal Variations in Laboratory Animal Tests Suggest That They Should Not be Considered Adverse

A 2007 publication by Carney and Kimmel reviews the underlying mechanisms for delayed ossification and other skeletal variations in laboratory animals and evaluates the

[8] Nemec MD. An inhalation two-generation toxicity study of methyl isobutyl ketone (MIBK) in rats. Report of Toxicology & Environmental Research and Consulting. The Dow Chemical Company. Midland, MI (2000). (The study is cited as WIL Research Laboratories (2000) in the IRIS assessment.)

different scenarios in which they have been observed.[9] The review notes that minor delays in ossification "seem to be readily repairable via postnatal skeletal remodeling and are not mechanistically linked to malformation." Carney and Kimmel also note that ossification delays often are seen In the presence of maternal or fetal toxicity. The authors conclude that "these minor variations would not generally be considered adverse in and of themselves but should be interpreted in the context of other maternal and fetal findings, information on normal skeletogenesis patterns, mode of action of the test agent, and historical control incidence." No such considerations were included in USEPA's IRIS assessment.

For the reasons outlined above, OEHHA cannot use the USEPA's 2003 IRIS assessment as a basis for listing MIBK as a reproductive toxicant under Proposition 65. The IRIS assessment does not consider important and scientifically valid data that clearly establish that the chemical does not satisfy the criteria for listing as a reproductive toxicant under Proposition 65.

Sincerely,

Steve Risotto

Stephen P. Risotto
Senior Director

Enclosure

[9] Carney EW and Kimmel CA. Interpretation of skeletal variations for human risk assessment: delayed ossification and wavy ribs. *Birth Defects Research* 80:473-496 (2007).

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May 14, 2013

Ms. Cynthia Oshita
Office of Environmental Health Hazard Assessment
State of California
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Sacramento, CA 95814

Re: Request for Relevant Information on Chemical Being Considered for Listing by the Authoritative Bodies Mechanism: Methyl Isobutyl Ketone, March 15, 2013

Dear Ms. Oshita:

The Ketones Panel of the American Chemistry Council submits the following information in response to the Office of Environmental Health Hazard Assessment's (OEHHA) request for information related to its consideration of listing of methyl isobutyl ketone (MIBK) as a reproductive toxicant under Proposition 65. The Ketones Panel represents US manufacturers of MIBK and other ketones.

The March 15, 2013 notice indicates that listing of MIBK is being considered under the authoritative bodies mechanism (Health and Safety Code Section 25306), based on the 2003 update of the US Environmental Protection Agency's (USEPA) Integrated Risk Information System (IRIS) assessment for MIBK. As part of this update, USEPA calculated a reference dose (RfD) for developmental effects in rats and mice exposed to a massive dose of MIBK in a study by Tyl *et al.,* 1987. 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.

Available Reproductive and Developmental Studies

The 2003 IRIS assessment indicates that there is no epidemiological evidence related to the potential for MIBK to cause reproductive or developmental effects. Consequently, USEPA's assessment focused on the results from the following two studies[1] in laboratory animals –

[1] Copies of these studies are included on the enclosed CD. The disk includes the raw data and analysis from the two studies.

- Inhalation two-generation reproductive toxicity study of methyl isobutyl ketone in Crl:CD®(SD)BR rats, and

- Developmental toxicity evaluation of inhaled methyl isobutyl ketone in Fischer 344 rats and CD-1 mice.

The Ketones Panel is not aware of any human or animal evidence on reproductive and developmental toxicity that has become available subsequent to USEPA's analysis.

Two-Generation Reproductive Toxicity Study (Nemec, 2000)

Nemec (2000) evaluated the reproductive toxicity of MIBK in a two-generation inhalation study in Crl:CD®(SD)BR rats. Groups of 30 male and 30 female F0 rats were exposed whole-body to MIBK vapors at mean measured concentrations of 0, 491 or 506, 999 or 1002, and 1996 or 2006 parts per million (ppm) – equal to 0, 2012 or 2073, 4093 or 4105, and 8178 or 8219 milligrams per m^3 or mg/m^3 – for 6 hrs/day for 70 consecutive days prior to mating, throughout mating, and for two generations. F0 and F1 parental rats were evaluated for reproductive endpoints, survival, clinical signs, startle response, food consumption, body weight, organ weights, comprehensive gross pathology, and histopathology of major organ systems and all gross lesions. F1 and F2 pups were evaluated for developmental endpoints, including postnatal survival, clinical signs, body weight, and external anatomical integrity. F1 pups were also evaluated for balanopreputial separation in males and vaginal perforation in females. Complete gross pathology evaluations were performed in F1 pups that were not selected for mating and in all F2 pups.

Nemec reported no reproductive or neonatal developmental effects in either generation of rats exposed to air concentrations of MIBK up to 8219 mg/m^3. The evaluation included the number of offspring with gross external malformations at birth, number of stillbirths, number of live births, body weight on postnatal day 1, and survival to postnatal day 4.

Developmental Toxicity Study (Tyl et al., 1987)

Developmental and maternal toxicity were evaluated by Tyl *et al.* (1987) in groups of 30 pregnant CD-1 mice and 35 pregnant Fischer 344 rats exposed by inhalation to 0, 300, 1000, or 3000 ppm (0, 1229, 4106, 12292 mg/m^3) MIBK for 6 hrs/day on gestation days 6 through 15. Animals were sacrificed on gestation day 18 (mice) or 21 (rats). Dams were evaluated for exposure-related changes in clinical signs, body weight, food consumption, organ weights (kidney, liver, and gravid uterus), and reproductive parameters. Fetuses were evaluated for exposure-related changes in body weight and viability, and for external, skeletal, and thoracic and peritoneal visceral alterations.

Developmental effects were limited to the highest dose group in both rats and mice and included a decrease in fetal body weight in both species (5.9 to 6.9% in rats; 10.2 to 13.9% in mice) and an increase in skeletal variations. 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). In mice, the significant maternal toxicity in the 12292 mg/m^3 group was manifested by the death of 3 dams (12% of the group) on the first day of exposure. In the rats, evidence of excessive maternal toxicity include clinical signs in the majority of animals (coordination loss, piloerection), body weight loss (GD 6-9), and 85% lower body weight gain (GD 6-12).[2] Also, the dams had a 5% lower body weight at termination relative to the control group.

Sufficiency of Evidence Presented in the IRIS Assessment

Section 25306(g) of the Health and Safety Code states that the criteria for reproductive toxicity shall be met if there exists a causal relationship between the chemical and reproductive toxicity "taking into account the adequacy of the experimental design and other parameters such as, but not limited to, route of administration, frequency and duration of exposure, numbers of test animals, choice of species, choice of dosage levels, and consideration of maternal toxicity." Section 25306(h) further notes that "a chemical does not satisfy the definition of 'as causing reproductive toxicity' if scientifically valid data which were not considered by the authoritative body clearly establish that the chemical does not satisfy the criteria."

OEHHA's November 1993 criteria for recommending listing as a reproductive toxicant[3] specify that the high dose level used in experimental animal tests "should elicit maternal toxicity," but do not provide guidance on what can be considered an appropriately high dose. USEPA's test guidelines for developmental toxicity studies,[4] on the other hand, do provide such guidance which appears to have been ignored in the 2003 IRIS assessment. 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."

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[2] Based upon high-dose selection for current regulatory guideline prenatal developmental toxicity studies targeting a 5-10% reduction in body weight gains, this dose level would clearly be considered excessively maternally toxic and unsuitable. [need citation]

[3] Available at http://www.oehha.ca.gov/prop65/policy_procedure/pdf_zip/dartCriteriaNov1993.pdf.

[4] USEPA. Health Effects Test Guidelines OPPTS 870.3700 Prenatal Developmental Toxicity Study. Office of Prevention, Pesticides and Toxic Substances. EPA 712-C-98-207 (August 1998). Available at http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series870.htm.

The highest dose employed in the study by Tyl *et al.* (1987) clearly exceeds both of the provisions of the USEPA guidelines. As indicated, 3 of the 36 mice dams (12%) in the study died after just one day of exposure to the highest dose and the rat dams exhibited excessively low weight gain during GD 6 through 12. The IRIS assessment briefly discusses the deaths among the mouse dams, 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.

The highest tested MIBK dose concentration (12292 mg/m^3) in the teratogenicity study was excessively high and should not be considered as a basis for Proposition 65 regulation. The study by Tyl *et al.* was conducted prior to the implementation of USEPA's test guideline. Had the study been performed after 1998, the researchers likely would have selected a much different dose range.[5] The test guidance 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.[6] 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.

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.

Sincerely,

Steve Risotto

Stephen P. Risotto
Senior Director

Enclosure

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[5] Even the middle dose of 4106 mg/m^3 in the study by Tyl *et al.* is above the inhalation limit concentration of 2 mg/L outlined by the relevant developmental toxicity guidelines (OPPTS 870.3700).

[6] Available at http://www.epa.gov/raf/publications/pdfs/DEVTOX.PDF.