MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

February 22, 2016

To: File for Tertiary-amyl methyl Ether (994-05-8)

From: Mike Depa, Toxics Unit, Air Quality Division

Subject: Initial Threshold Screening Level

The Initial Threshold Screening Level (ITSL) for tertiary-amyl methyl ether is 62 µg/m^3 with annual averaging time.

The previous ITSL was 62 µg/m^3 with a 24-hr averaging time (see the attached memo from Marco Bianchi, dated April 26, 2001). The current file review concludes that the averaging time for the chronic reference concentration (RfC) derived ITSL may appropriately be set at annual, based on the nature and duration of the key study and the ITSL value derivation, as allowed under Rule 229(2)(b).

MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

April 26, 2001

To: File for Tertiary-amyl methyl Ether (994-05-8)

From: Marco Bianchi, Toxics Unit, Air Quality Division

Subject: Initial Threshold Screening Level

The Initial Threshold Screening Level (ITSL) for tertiary-amyl methyl ether (TAME), is 62 µg/m^3 based on a 24-hr averaging time. The following references or databases were searched to identify data to determine the ITSL/IRSL: IRIS-online, HEAST, NTP Management Status Report-online, RTECS, EPB-CCD, EPB library, CAS-online, NLM-online, IARC-online, NIOSH Pocket Guide, and ACGIH Guide.

Tertiary-amyl methyl ether is known as an oxygenated fuel compound, or a chemical component that adds oxygen to gasoline. Oxygenated fuel is used during winter months in designated areas of the country with high levels of carbon monoxide pollution. Exposure of the general public to oxygenates can occur during automobile refueling, while in the vehicle, or while at the perimeter of a service station. RTECS has listed an oral rat LD of 1602 mg/kg and an inhalation rat LC50 of >5400 mg/m^3.

4-week subacute and neurotoxicity assessment

In the 4-week subacute study, 14 Sprague-Dawley rats/sex/group were exposed by inhalation to TAME vapor at target concentrations of 0, 500, 2000, or 4,000 ppm, 6 hours/day, 5 days/week for 4 weeks (Amoco Corp. 1992). A functional observational battery (FOB) which evaluated neuromuscular function, reflex response and sensory perception was performed one week prior to exposure and after 1, 5, and 20 exposures. To differentiate between transient central nervous system (CNS) depression and persistent neurotoxic sequelae, the FOB was performed on 4 rats/sex/group following exposure. Seven of 28 rats died as a result of exposure at 4000 ppm. Transient signs of CNS depression and concomitant changes in body temperature, hind limb splay, rotorod performance, tail pinch and righting reflex were observed at 4000 and/or 2000 ppm. Mean body weights/body weight gains were significantly decreased in the 4000 ppm exposed males. Select absolute and relative organ weight changes were detected. Affected clinical pathology parameters included alanine aminotransferase, aspartate aminotransferase, cholesterol, and triglycerides. No histopathologic findings were associated with the affected clinical chemistry or organ weight parameters. Thus, repeated exposure to TAME resulted in 25 percent mortality at 4000 ppm and produced transient signs of CNS depression with consequent transient changes in select FOB parameters at 2000 and 4000 ppm; organ weight and clinical

chemistry changes were observed without underlying histopathology. The results indicated that 500 ppm was a no- observable-adverse-effect-level (NOAEL) for TAME.

Developmental inhalation toxicity study - rats and mice

In a developmental inhalation toxicity study, time-pregnant CD (Sprague-Dawley rats were exposed to TAME vapor 6 hours/day on gestational days 6-19 at target concentrations of 0, 250, 1500 or 3500 ppm (API, 1997a). Similarly, CD-1 mice were exposed to 0, 250, 1500, 3500 ppm, 6 hours/day, but on gestational days 6-16 (API, 1997b). Maternal body weights, weight changes, feed consumption, and clinical observations were documented throughout gestation for gestation days (gd) 0-20 for rats, and gd 0-17 for mice. At scheduled necropsy maternal body, liver and gravid uterine weights were recorded; ovarian corpora lutea and uterine implantation sites (resorptions, dead and live fetuses) were counted. Live fetuses were examined for sex weight, external, visceral (including craniofacial) and skeletal alterations.

For rats, no dams died, aborted or delivered early. Maternal body weights were significantly reduced at 3500 ppm; maternal weight gains were significantly reduced at 1500 and 3500 ppm. Maternal liver weight relative to terminal body weight was significantly increased at 3500 ppm. Treatment-related clinical observations included ataxia, dazed appearance, lethargy, eyes squinted or closed, and slow respiration at 3500 ppm, and lethargy and piloerection at 1500 ppm. Maternal feed consumption was significantly reduced at 1500 and 3500 ppm during the exposure period. Developmental toxicity was present at 3500 ppm, which significantly reduced fetal body weight per litter. There were no treatment-related changes in the incidence or severity of fetal external, visceral, skeletal or total malformations or variations in this study. The NOAEL for rat maternal toxicity was 250 ppm and for developmental toxicity was 1500 ppm in rats under the conditions of this study.

For mice, four dams (of 25) died at 3500 ppm, one each on gravid day (gd) 6, 7, 8, and 9. One dam at 250 ppm delivered early. Maternal body weights and body weight gains were significantly reduced at 3500 ppm. Maternal liver weights, absolute and relative to terminal body weight were significantly increased at 1500 and 3500 ppm. Treatment-related clinical observations included mortality, ataxia, prone positioning, gasping, rough coat, lethargy, eyes squinted, head tremors, and slow respiration at 3500 ppm, and eyes half closed and head tremors (one dam each) at 1500 ppm. Maternal feed consumption was significantly reduced at 1500 and 3500 ppm during the exposure period. Developmental toxicity was present at 3500 ppm, specifically significant increased incidence of late fetal deaths, significantly reduced fetal body weights per litter, and increased incidences of cleft palate (external malformation) and enlarged ventricles of the cerebrum (a visceral variation). At 1500 ppm, fetuses also exhibited an increased incidence of cleft palate. The NOAEL for mice maternal and developmental toxicity was 250 ppm in mice under the conditions of this study.

Two-generation reproductive toxicity evaluation

In a two-generation reproductive toxicity evaluation, CD Sprague-Dawley rats, 30/sex/group designated F0 generation, were exposed to TAME vapor at 0, 250, 1500, and 3000 ppm for 6 hrs/day, for a 10-week pre-breed exposure period, mating, gestation and lactation; the offspring (designated F1), 30/sex/group, were similarly exposed through lactation (API, 1998). Their offspring (designated F2), 30/sex/group were retained after weaning with no exposure to TAME until acquisition of vaginal patency (females) or preputial separation (males). Parental F0 and F1

animals were necropsied with selected organ weights, histopathologic examination of reproductive organs and andrological assessments; selected F1 and F2 weanlings, up to three/sex/litter, were necropsied with selected organ weights. Vaginal cyclicity was assessed for the last three weeks of the pre-breed exposure period for F0 and F1 females. Anogenital distance was measured in F2 pups on the clay of birth. Adult systemic toxicity was present in F0 and F1 parents at 1500 and 3000 ppm. Scientific findings at one or both exposure-concentrations in one or both generations included transient ataxia, and reductions in body weight gain and feed consumption. At necropsy, there were changes in absolute and relative organ weights for the liver, kidney, adrenals, and spleen; however, there were no treatment-related histopathological findings in these tissues. Equivocal findings of adult reproductive toxicity were present in F0 and F1 males at 3000 ppm. These findings consisted of reduced epididymal sperm counts in F1 males at 3000 ppm and an increased percentage of abnormal sperm in F0 males at 1500 and 3000 ppm. These findings were not consistent across generations and the percent values for abnormal sperm were within the historical control range. There were no effects on other measures of male reproductive function. Therefore the findings on sperm morphology were considered to be of doubtful toxicological significance. No treatment-related effects on female reproductive function were found. Offspring toxicity was present in both males and females in the F1 and F2 generations at 1500 and 3000 ppm. Findings included reduced pup body weights, delayed acquisition of vaginal patency and preputial separation, and shorter anogenital distance in F2 offspring at 3000 ppm (associated with reduced body weights). F2 survival indices were also reduced on post-natal day (pnd) 4 and pnd 21 at 3000 ppm. The NOAEL for adult systemic toxicity and offspring toxicity was 250 ppm. The NOAEL for adult reproductive toxicity was 1500 ppm.

13-week study

In the 13-week inhalation study, male and female Fischer-344 rats and CD-1 mice were exposed to 0, 250, 1500, or 3500 ppm of TAME for 6 hour/day, 5 days/week over a 13-week period (API, 1997c). There were 51 rats/sex/group in the control and high-exposure groups and 41 rats/sex/group in the low- and mid-exposure groups. For mice, there were 46 animals/sex/group in the control and high-exposure groups and 36 animals/sex/group in the low- and mid-exposure groups. Because of the high incidence of mortality in mice exposed to 3500 ppm, a new high-exposure group of mice at 2500 ppm was established together with a concomitant air control group.

There was a low incidence of mortality in the high-exposure group. Abnormal clinical signs, mainly prostration and lethargy, were seen during exposure in the high- and mid-exposure groups. Body weights and body weight gains were decreased in the high- exposure group. There were increased platelet counts in the high-exposure males and females, and mid-exposure males. Serum levels of total protein, albumin and globulin were increased in the high- and mid-exposure groups. Absolute liver weights, and liver to body weight and liver to brain weight ratios were increased in high-exposure and mid-exposure males and females, and low-exposure males. Increases in absolute and/or relative kidney weights were found in the high-exposure males and females and mid-exposure females. There were decreases in absolute brain weight and heart to body weight ratio in the high-exposure males and females. Most of these effects were reversible after the 4-week recovery period with the exception of the decrease in body weight, body weight gain, and brain weight in the high-exposure males. Microscopic findings in the nasopharynx and nasoturbinal tissues included hypertrophy/hyperplasia of goblet cells (minimal to moderate) in the

respiratory mucosa of rats from the control to high-exposure groups. In both the nasoturbinal tissues and in the nasopharynx, goblet cell hypertrophy/hyperplasia was considered to be a localized adaptive response to a minimal irritant effect rather than an adverse toxicological response to the test material. Based on these findings, the NOAEL for female rat exposure to TAME for 13 weeks was 250 ppm; a NOAEL for male rats exposure was not established. Exposure to 250 ppm resulted in an increase in the absolute liver weight and liver to body weight and liver to brain weight ratios in males only.

Neurobehavioral evaluation of rats provided evidence of neurological effects of TAME (depression of CNS activity and neuromuscular impairment) in high-exposure males and females, and mid-exposure males one hour after acute exposure; these effects were no longer evident 6 and 24 hours after acute exposure and were not seen after repeated exposure to the test material for the remainder of the study. There were no neuropathological changes at any exposure level. The NOEL for acute neurobehavioral effects of TAME was 250 ppm in males and 1500 ppm in females.

In mice exposed to TAME, mortality was observed in the high-exposure group. Prostration, lethargy and/or decreased activity were found in the high-exposure mice during and immediately following exposure; lethargy and some prostration were seen in the mid-exposure group. There were slight effects on clinical chemistry parameters in high-exposure males and females (increases in alanine aminotransferase activity and blood urea nitrogen in females at week 14, increases in total protein, albumin and globulin in males at week 5 and increases in globulin in females at week 5) and mid-exposure males (increase in globulin at week 6). There were increases in absolute liver weight, and liver to body weight and liver to brain weight rations in the high-exposure males and females, and mid-exposure males. Cell proliferation studies in the liver showed an increase in the labeling index of hepatocytes in high-and mid-exposure males and females, and low-exposure females. There was microscopic evidence of centrilobular hepatocellular hypertrophy in high- and mid-exposure males and females. Based on these findings the NOEL for male mice exposed to TAME was 250 ppm; a NOEL for female mice was not established. Whole body exposure to 250 ppm resulted in an increased labeling of index of hepatocytes for female mice only.

Derivation of the Reference Concentration

The quality of the 13-week inhalation study used to evaluate TAME justifies deriving a reference concentration (RfC) for this compound according to Rule 232(1)(a). The study was of sufficient duration and used an appropriate amount of test animals. Clinical chemistry, gross and pathologic examination and a neurological assessment were used to evaluate the outcome of the TAME inhalation exposures. In addition, a developmental and a two-generation reproductive study produced similar results and provide supporting information to establish an RfC. Therefore, the 13-week study (API, 1997c) is appropriate to use for an RfC derivation. A Lowest-Observed-Adverse-Effect-Level (LOAEL) of 250 ppm was identified based on increase in the absolute liver weight and liver to body weight and liver to brain weight ratios in male rats and an increased labeling of index of hepatocytes for female mice.

It was determined that TAME is a Category 3 gas as it is relatively water insoluble and unreactive in the extrathoracic and tracheobronchial regions. The site of toxicity was generally remote to the

pulmonary region. Study results indicated that this compound affected the CNS causing a number of clinical effects, e.g., ataxia, slow respiration, head tremors, etc.

Conversion from ppm to mg/m^3

Molecular Weight of ETBE = 102.2 g

LOAEL = 250 ppm

mg/m^3 = ppm x MW/24.45

mg/m^3 = 250 ppm x 102.2/24.45

mg/m^3 = 1043 mg/m^3

Adjust for Exposure Regimen

$LOAEL_{ADJ}$ = E (mg/m^3) x D (h/24h) x W (days/days)

E = experimental dose level

D = number of hours exposed/24 h; and

W = number of days of exposure/7 days

$LOAEL_{ADJ}$ = 1043 mg/m^3 x 6h/24h x 5days/7days

$LOAEL_{ADJ}$ = 186 mg/m^3

Dosimetric Adjustments and Calculation of $NOAEL_{hec}$

The human equivalent concentration (HEC) is used to derive the RfC. The HEC is calculated as:

$LOAEL_{HEC}$ = $LOAEL_{ADJ}$ x DAF

Where DAF = dosimetric adjustment factor

For category 3 gases the DAF is the ratio of animal blood:gas (air) partition coefficient (Hb/g) to human Hb/g. If the Hb/g is unknown then the default value of 1 is used. The values for animals and human Hb/g are unknown, therefore, the $LOAEL_{HEC}$ = $LOAEL_{ADJ}$.

Finally, the RfC is calculated as:

RfC = $LOAEL_{HEC}$/(UFxUFxUF)

Where UF = Uncertainty Factor

RfC = (186 mg/m^3)/3x10x10x10 = 0.062 mg/m^3

Where

3 = interspecies extrapolation

10 = to account for sensitive individuals

10 = subchronic to chronic study

10 = for LOAEL to NOAEL extrapolation

RfC = 0.062 mg/m^3 x 1000µg/mg = 62 $µg/m^3$

Reference:

1. API (American Petroleum Institute). 1998. Final report, two-generation reproductive toxicity evaluation of inhaled tertiary amyl methyl ether (TAME) vapor in CD (sprague-dawley) rats, with cover letter. Toxic Substance Control Act (TSCA) 8(e) submittal; 42180 Fi-022 44648 (0TS0559331).

2. Amoco Corp. 1992. Initial submission: four-week inhalation study of tert-amyl methyl ether in rats including neurotoxicity assessment (poster presentation format) with cover letter. Toxic Substance Control Act (TSCA) 8(e) submittal; 8EHQ-0292-2299 (0TS053571 1).

3. API (American Petroleum Institute). 1997a. Developmental toxicity evaluation of inhaled tertiary amyl methyl ether (TAME) in CD (sprague-dawley) rats, with cover letter. Toxic Substance Control Act (TSCA) 8(e) submittal; 42180 Fi-15 44639 (0TS0558879).

4. API (American Petroleum Institute). 1997b. Developmental toxicity evaluation of inhaled tertiary amyl methyl ether (TAME) in CD-mice, with cover letter. Toxic Substance Control Act (TSCA) 8(e) submittal; 42180 Fi-1644639 (0TS0558880).

5. API (American Petroleum Institute). 1997c. A 13-week inhalation toxicity/neurotoxicity study of tertiary amyl methyl ether (TAME) in the rat and mouse via whole-body exposure with a 4- week recovery period. Toxic Substance Control Act (TSCA) 8(e) submittal; 42180 F1-20 44643 (0TS0558892).