MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

TO: File for t-Butanol (CAS # 75-65-0)

FROM: Robert Sills, AQD Toxics Unit Supervisor

SUBJECT: t-Butanol change in the averaging time from 24 hrs to annual

DATE: December 22, 2016

The ITSL for t-butanol is 1890 ug/m^3, with annual averaging time.

The ITSL for t-butanol (1890 ug/m^3) was established on April 15, 1998 (see attached). The averaging time (AT) assigned to the ITSL at that time was 24 hours, as per the default methodology at that time (Rule 232(2)(b)). The ITSL was based on a chronic animal bioassay. A total uncertainty factor (UF) of 1000 was applied, which consisted of a UF = 10 for interspecies extrapolation, UF = 10 for intraspecies variability, and UF = 10 for LOAEL-to-NOAEL conversion. The current file review concludes that the AT for the 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). Therefore, the averaging time is being changed from 24 hrs to annual.

MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

April 15, 1998

TO: File for t-butyl alcohol (CAS #75-65-0)

FROM: Marco Bianchi, Toxics Unit, Air Quality Division

SUBJECT: Initial Threshold Screening Level

The Initial Threshold Screening Level (ITSL) for t-butyl alcohol is 1,890 ug/m^3 based on an 24 hr. averaging time. This compound was initially evaluated by AQD staff in 1985, using pre-Rule 230 procedures to derive an impact of 3000 ug/m^3 with an 8 hr averaging time. This value was grandfathered into the interim ITSL/IRSL list established in 1992. In an effort to finalize all interim chemical screening levels, this chemical was re-reviewed to set a final ITSL/Initial Risk Screening Level (IRSL). The following references or databases were searched to identify data to determine the ITSL/IRSL: IRIS, HEAST, NTP Management Status Report, RTECS, EPB-CCD, EPB library, CAS-online, NLM-online, IARC, NIOSH Pocket Guide, ACGIH Guide, and the EPA's Science Advisory Board's draft document, Assessment of Thyroid Follicular Cell Tumors.

The chemical t-Butyl alcohol is used in perfumes and a variety of cosmetic and cleaning products. It is a colorless, volatile liquid with a camphor-like odor, and considered a nonmetabolized alcohol, since it cannot form an aldehyde or ketone by dehydrogenation nor used as a substrate for alcohol dehydrogenase. Tertiary alcohols are metabolized slowly and incompletely, so their toxic effects are especially persistent. Most of the tertiary alcohols are central nervous system depressants.

The oral LD_{50} of t-butanol has been reported as 3.5 g/kg in rats, and 3.6 g/kg in rabbits. The reported LD_{50} for mice by intraperitoneal injection is 441 mg/kg. The primary acute effects in animals are signs of alcoholic intoxication.

In some human individuals, t-butanol is a mild skin irritant. No other adverse effects of t-butanol on humans were found in the literature, including effects from reproductive/development, inhalation and carcinogenicity studies. However, because of its wide use in commercial preparations and its presence in drinking water, the National Cancer Institute nominated t-butanol for further study.

The National Toxicology Program issued a technical report on the carcinogenicity of t-butanol in 1995. A review of the scientific literature indicated this to be the only study of sufficient duration to assess carcinogenic potential. In this study, groups of 60 male and 60 female mice were exposed to 0, 5, 10, or 20 mg/mL of t-butanol in drinking water for two years. The resulting average daily doses were 535, 1,035 or 2,065 mg/kg for male mice, and 510, 1,015, or

2,105 mg/kg for female mice. Survival in high dose males was significantly lower than controls but was unaffected in all other groups of males and all groups of females. Mean body weight was significantly depressed due to decreased water consumption (taste aversion) in high dose females. The incidence of thyroid gland follicular cell hyperplasia was significantly increased in all groups of dosed males and in mid-and high-dose females. The incidence of follicular cell adenoma and carcinoma combined was marginally increased in mid-dose males. The incidence of follicular cell adenomas was significantly increased in high dose females. It was concluded that there was some evidence of carcinogenic activity of t-butanol in female mice and equivocal evidence in male mice.

In addition to the studies in mice, groups of 60 male rats were exposed to average daily doses of 85, 195, or 420 mg/kg, and females at 175, 330 or 650 mg/kg of t-butanol in drinking water. Incidence of focal renal tubule hyperplasia and of adenoma were significantly increased in all groups of exposed males. Carcinomas occurred in mid and high-dose males but the incidence was not significantly elevated above controls. The severity of nephropathy and incidence of and severity of transitional cell hyperplasia of the kidney were increased in exposed male and female rats. Based on these findings, it was concluded that there was no evidence of carcinogenic activity of t-butanol in female rats and there was some evidence in male rats.

The renal effects seen in male and female rats were characterized by an exacerbation of the changes commonly seen in control rats at the end of a 2-year study. However, the observed mineralization in the renal medulla of exposed males is a common feature reported for hyaline droplet nephropathy. This effect appears to be species and sex specific and has not been reported to occur in human males and in females of any species. While some supporting evidence of nephropathy was observed in female rats in this study, overall, the data does not support either the development of an oral slope factor or an oral reference dose based on renal effects in rats. Therefore, the screening level for t-butanol will be based on NTP data from the 2-year mice study.

Although the NTP study for t-butanol does show *some evidence* of mouse thyroid follicular cell adenomas, uncertainties exist as to whether this compound truly exerts this effect in a non-threshold manner. This is because the EPA's Scientific Advisory Board (SAB) has concluded that there may be sufficient evidence to support a threshold mechanism which is likely to apply to the development of certain thyroid follicular tumors. Tumors arise under conditions in which there is prolonged decrease in circulating thyroid hormone and increase in the thyroid stimulating hormone (TSH). Under continued TSH stimulation, thyroid follicular cells undergo hypertrophy, hyperplasia, and eventually, neoplasia. Based on this evidence, the SAB developed a policy for risk assessment of agents that cause thyroid cell tumors:

> *Threshold models may be applied in dose-response assessments for those chemical substances where only thyroid tumors (and relevant pituitary tumors) have been produced; the tumors can be*

attributed to a disruption in thyroid-pituitary imbalance, e.g., genotoxicity, can be ruled out. Where there are tumors at other sites and/or genotoxicity is present, it is presumed that threshold models will not be used; however, case-by-case determinations are possible. Threshold models will not be used where there is no evidence of thyroid-pituitary imbalance.

Most of the focus in implementing this policy is devoted to answering the following questions: (a) Does an agent that shows thyroid carcinogenic effects have antithyroid activity; (b) Can modes of action other than thyroid-pituitary disruption account for thyroid tumor formation by this chemical; and (c) How can one express thyroid dose-response relationships? To determine whether a carcinogen works by way of a threshold mechanism on thyroid follicular cells, a determination of the antithyroid activity of a chemical *requires* empirical demonstration of the following points: (1) thyroid growth, (2) thyroid and pituitary hormone changes, (3) location of the site(s) of antithyroid action, and (4) dose correlation among effects. An evaluation will be made as to whether a threshold mechanism exists for t-Butanol exposure by evaluating if these points were present in the 2-year mouse study. Each of these points will be rated *favorable* - suggesting threshold characteristics; or *unfavorable* - suggesting non-threshold characteristics. In addition, the data will be rated low, medium or high, as to whether it empirically satisfies each of the above requirements. Then a determination will be made based on these ratings as to whether t-butanol is acting by way of a threshold or non-threshold mechanism.

- ***thyroid growth*** *- agents which effect thyroid-pituitary functioning stimulate thyroid enlargement. Commonly measured parameters include but are not limited to increases in absolute or relative thyroid gland weight or to histological indications of cellular hypertrophy and hyperplasia, morphometric documentation of alteration in thyroid cellular components and changes in the proliferation of follicular cells detected by DNA labeling or mitotic indices.*

  In the NTP study, the incidences of thyroid follicular cell hyperplasia were significantly increased in all groups of exposed male mice and in 10 and 20 mg/mL female mice.

  Rating: favorable; quality of data: high. *Thyroid follicular cell hyperplasia occurred in all but one dose group of mice.*

- ***hormone changes*** *- with a disruption in thyroid-pituitary functioning, there is typically a reduction in both circulating serum T4 and T3 concentrations and an increase in TSH levels within days or a few weeks of chemical administration.*

  Blood tests to determine hormone homeostasis was not performed for the 2-year drinking water study. Therefore, it can not be determined if T3 and T4 concentrations were effected. Hematology, urinalysis and clinical chemistry were conducted in rats and mice for a 15 month interim evaluation and in 13-week

drinking water studies, but these data could not be used to infer any hormonal changes from t-butanol exposure.

The only evidence to suggest that a disruption in thyroid-pituitary functioning may be occurring is indirect evidence. In the NTP study, t-butyl alcohol was tested for induction of genetic damage both *in vivo* and *in vitro* (with and without metabolic activation). These tests included, Salmonella typhimurium, mouse lymphoma, sister chromatid exchange, Chinese hamster ovary cells, and mouse micronucleated erythrocytes. **All results were negative.** Therefore, genotoxicity may be ruled out leaving thyroid-pituitary dysfunction a plausible mechanism of action.

Rating: questionable; quality of data: low. *There is no hard evidence that thyroid/pituitary hormonal changes have occurred, but only because analytical tests haven't been performed to determine this condition. There is direct evidence that the mechanism of action is not due to genotoxicity, suggesting a perturbation in metabolism.*

- ***site of action*** *- chemicals that produce thyroid tumors alone or after administration of a mutagenic initiator produce interference with thyroid-pituitary function by a variety of specific means. Effects have been found at one or more of the following anatomical locations - intrathyroidal and various extrathyroidal sites, including the liver and possible other sites. For example, a significant amount of thyroid hormone is normally metabolized by the liver. Certain chemicals induce microsomal enzymes and enhance thyroid hormone metabolism and removal. T4 conjugation with glucuronic acid is enhanced by those agents that induce glucuronyl transferase. Common manifestations of microsomal induction include such things as enlargement of hepatocytes in the centrolobular region, increase in hepatic cell smooth endoplasmic reticulum, increase in P-450 associated metabolism of various chemical substrates, and increase biliary flow.*

  The possible routes of metabolism of tertiary alcohols are direct conjugation of the hydroxyl group with glucuronic acid and oxidation of one or more of the alkyl substituents (Williams, 1959). Early metabolism studies identified glucuronide conjugates of t-butyl alcohol in the urine of rabbits (Kamil et al., 1953). Approximately 24% of the single gavage dose of 4 mmol/kg was excreted as glucuronide.

  After five days of inhalation exposure to 500 ppm, t-butyl alcohol caused a 36% increase in microsomal cytochrome P450 in the kidneys of male Sprague-Dawley rats; liver and lung P450 were unaffected. After 3 days of inhalation exposure to 2,000 ppm t-butyl alcohol, hepatic P450 was significantly elevated 28%; kidney P450 was not affected, and lung P450 was slightly decreased. These results may indicate that a longer duration of exposure is required before induction can be observed in the kidney, whereas concentration may be more important for induction

in the liver (NTP, 1995). These findings also suggest a threshold mechanism may be involved.

Rating: favorable; quality of data: medium-high. *Metabolic studies have shown glucuronic conjugates of t-butanol, and elevation of P-450 enzymes in the liver at what appears to be, a threshold level.*

- ***dose correlations -*** *confidence in an antithyroid mode of action is enhanced by evidence of a correlation between doses of a chemical that do and do not jointly perturb thyroid/pituitary hormone levels, produce various histological changes in the thyroid and/or produce other effects, including thyroid cancer. These are important steps in evaluating the significance of thyroid-pituitary disruption in thyroid carcinogenesis and in evaluating dose-response relationships.*

Table 1. Incidence of neoplasms of the thyroid gland in mice (2 yr. drinking water study)

	Cell Type	0 mg/mL	5 mg/mL	10 mg/mL	20 mg/mL
Male	follicular cell hyperplasia	8.3%	30.5%	25.4%	31.6%
	follicular cell adenoma	2.0%	0.0%	7.0%	2.0%
	follicular cell adenoma or carcinoma	2.0%	0.0%	7.0%	4.0%
Female	follicular cell hyperplasia	32.8%	46.7%	55.9%	79.7%
	follicular cell adenoma	3.0%	5.0%	3.0%	15.0%

In a 13-week drinking water study, both rats and mice of each sex were exposed to 0, 2.5, 5, 10 and 20 mg/mL of t-butanol. After a complete histological examination which included examining the pituitary and thyroid gland, there was no evidence of any effect including weight changes or hyperplasia. Comparatively, in the 2 yr drinking water study, rats had no incident of thyroid follicular cell effects. However, for the mice (Table 1), the incidence of follicular cell adenoma was marginally increased in 10 mg/mL males and a follicular cell carcinoma was present in one male in the 20 mg/mL group. In 20 mg/mL female, the incidence of follicular adenoma was significantly greater than that of the controls and occurred bilaterally in one female in this exposure group. The incidences of follicular cell hyperplasia were significantly increased in all groups of exposed male and in 10-20 mg/mL females. The increased incidence of follicular cell adenoma in 20 mg/mL female mice was statistically significant, and the rate of 15% exceeds the maximum rate of 5% observed in controls in previous NTP drinking water studies. In addition, there were concomitant significant increases in incidences of follicular cell hyperplasia in the 10 and 20 mg/mL females. These findings in female mice constitute some evidence of

carcinogenic activity for t-butyl alcohol. Although male mice did not demonstrate a significant increase in the incidence of benign or malignant follicular cell neoplasms, the incidence of follicular cell hyperplasia was significantly increased in all exposed groups. The incidence of adenoma of 7% in 10 mg/mL male mice, while statistically significant, exceeds the maximum rate of 2% observed historically in untreated NTP drinking water controls, and the incidence of 4% in the 20 mg/mL group may have been related to the reduced survival in this group (Table 1.).

Rating: favorable; quality of data: medium. There appears to be some correlation between doses of t-butanol that do and do not produce histological changes in the thyroid.

- ***lesion progression -*** *evidence for a progression of histological lesions over time following exposure to an agent, including cellular hypertrophy and hyperplasia, focal hyperplasia and neoplasia (benign and possibly malignant tumors).*

  According to the NTP study, "proliferation of thyroid gland follicular cells is generally considered to follow a progression from hyperplasia to adenoma and carcinoma." This was shown in the drinking water study by the incidence of neoplasms of thyroid follicular cells for both male and female mice as noted in Table 1. Lesion development did progress from follicular cell hyperplasia to follicular cell adenoma and carcinoma.

  Rating: favorable; quality of data: medium. There appears to be some evidence of follicular cell lesions progressing from hyperplasia to adenoma and carcinoma.

Individually, the study results don't provide unanimous proof that t-butanol acts by a threshold mechanism. But taken as a whole, these factors enhance the weight of evidence with only minor uncertainties, that carcinogenicity of t-butanol exposure acts by way of a threshold mechanism. Three out of the four empirical points listed above imply threshold characteristics, showing favorable results with medium to high confidence in the data. Additionally, supportive evidence of lesion progression also lends support to a threshold mechanism. For the remaining point, some uncertainty exists in the absence of measured T3 or T4 hormone levels, but only because the test was never performed in the NTP assay, not because it was measured but failed to increase in concentration. Therefore, an increase in T3 and T4 hormones may actually occur after t-butanol exposure. Because the preponderance of evidence suggests t-butanol acts by a threshold mechanism, it seems appropriate to use a threshold-based methodology to determine an air toxics screening level such as an EPA provisional reference dose (RfD). Since no EPA provisional [RfD] exists for t-butyl alcohol, this value will be derived according to the Air Quality Division's Toxic Rules 232(1)(b).

The critical effect in the NTP bioassay was determined to be follicular cell hyperplasia in male mice. Although hyperplasia is a pathological condition that is reversible, this study showed that it

progressed to follicular cell adenoma and carcinoma. Therefore, the RfD is based on a LOAEL of 535 mg/kg/day (adjusted average daily dose for low-dose group) for follicular cell hyperplasia of the thyroid in male mice. The application of a 1000 uncertainty factor (10 for interspecie differences, 10 for the extrapolation from animals to humans, and 10 to account for the use of a LOAEL) results in an oral RfD of 0.54 mg/kg/day. The RfD is considered to be protective of the threshold carcinogenic effects observed for t-butanol.

The ITSL was determined as follows:

LOAEL = 535 mg/kg/day (adjusted average daily dose)

Uncertainty factors:
Interspecie differences = 10
Animal to human extrapolation = 10
LOAEL to NOAEL = 10

$$\frac{535 \text{ mg/kg/day}}{10 \times 10 \times 10} = 0.54 \text{ mg/kg/day}$$

RfD = 0.54 mg/kg/day

$$\text{ITSL} = 0.54 \text{ mg/kg/day} \times \frac{70 \text{ kg}}{20 \text{ m}^3} = 1.89 \text{ mg/m}^3$$

$$1.89 \text{ mg/m3} \times \frac{1000 \text{ ug}}{1 \text{ mg}} = 1{,}890 \text{ ug/m}^3$$

The ITSL for t-butyl alcohol = 1,890 ug/m^3 based on 24 hr. averaging time.

References:

Michigan Department of Environmental Quality - Environmental Response Division. 1995. Justification Document for t-Butyl Alcohol.

National Toxicology Program. 1995. NTP Technical Report on the Toxicology and Carcinogenesis Studies of t-Butyl Alcohol (CAS NO. 75-65-0) in F344/N Rats and B6C3F$_1$ Mice (Drinking Water Studies). U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. NTP TR436. NIH Publication No. 95-3167.

Risk Assessment Forum. 1996. Assessment of Thyroid Follicular Cell Tumors. Scientific Advisory Board Review Document - Draft. *(Please note, although this document is in draft form and has no publication numbers, there is no indication that information from this document should not be quoted or cited.)*

MB:SLB
cc: Mary Lee Hultin, AQD