MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

JULY 15, 1996

TO: File for Triethylene Tetramine (CAS# 112-24-3)

FROM: Michael Depa, Toxics Unit

SUBJECT: Screening Level Determination

The initial threshold screening level (ITSL) for triethylene tetramine (TETA) is 8 $\mu g/m^3$ based on annual averaging time. A developmental ITSL has also been established at 3,675 $\mu g/m^3$ based on a 24-hour averaging time.

The following references or databases were searched to identify data to determine the ITSL: IRIS; RTECS; ACGIH Threshold Limit Values; NIOSH Pocket Guide to Hazardous Chemicals; Environmental Protection Bureau Library; IARC Monographs; CAS Online (1967 - November 30, 1995); National Library of Medicine; Health Effects Assessment Summary Tables; and NTP Status Report. Review of these sources found that EPA has not established an RfC or RfD for TETA. Occupational exposure limits were not available.

TETA causes systemic effects when absorbed, of an inflammatory, hepatotoxic nature, and also has mutagenic and teratogenic and reproductive effects potential (CHIP, 1982). However, TETA primarily effects the skin and lungs resulting in irritation and sensitization with subsequent pathologies such as asthma, finger-nail involvement, eczema and other dermatoses (CHIP, 1982).

In a human study, investigators analyzed the allergic reaction of TETA on a 54-year-old man who had worked as an aircraft fitter for 5 years (Fawcett et al, 1977). He first noticed occasional "flu-like" symptoms of runny nose, headache and watery eyes about 5 hours after starting the shift but no chest symptoms until 6 months later when he had his first attack of asthma one evening after the work shift. He did not experience asthma during weekends. Five months after the onset of symptoms the TETA process was withdrawn by the company and he had no further attacks of asthma. An exposure test was performed to analyze the effect. A mixture of Bisphenol A resin and TETA (curing agent) were brushed on a wooden board for 4 consecutive periods of 30 minutes with 5 minute intervals to measure respiratory function. The same man was pretested with exposure to only the Bisphenol A epoxy resin. Exposure to the epoxy resin and TETA mixture caused flue-like symptoms with headache and watering of eyes and nose

lasting for 1 hour after cessation of exposure. There was a gradual fall in FEV_1 during the first 4 hours and he became audibly wheezy. The FEV_1 remained low for 5 hours after exposure and did not return to pretest values until 7 hours after exposure. No changes in FEV_1 were observed when epoxy resin alone was used. No attempt to quantitate the exposure concentration was reported.

In another human study, 1200 mg/day (400 mg, 3 times daily) of TETA was given orally to 4 patients with primary biliary cirrhosis for a period ranging from 2 days to 20 weeks (Epstein et al., 1980). The treatment was discontinued in each case because of adverse effects. After two weeks a 56-year-old woman developed gastric pain and tenderness. After 20 weeks she developed a skin rash and a marked increase in epigastric pain and tenderness. She required hospitalization. Laboratory investigation revealed hypochromic anemia and gastric ulcers. A liver biopsy showed no histologic or copper concentration change from pretreatment results. A 35-year-old woman complained of cramps in both hands after 1 day of treatment. On the second day she developed severe muscle pain in the shoulder and pelvic groups. Full blood count, urea and electrolytes remained normal, but aspartate transaminase and creatinine phosphokinase levels rose significantly. Myoglobinuria was detected, confirming a diagnosis of acute rhabdomyolysis. Assuming 70 kg as the default body weight, a 2 day oral LOAEL of 17 mg/kg was identified from this study.

An oral LD50 study was found. Groups of 5 Sherman rats were given 1, 2, 4, or 8 g/kg of TETA and observed for 14 days (Smyth et al., 1949). The LD50 was determined to be 4.34 g/kg (3.81 - 4.94) by the method of Thompson. An ITSL was developed according to Rule 232(1)(h) and is described below.

$$ITSL = 1/500 \times 1/40 \times 1/100 \times LD50/0.167 \times W_a/I_a$$

$$ITSL = 1/500 \times 1/40 \times 1/100 \times (4{,}340\ mg/kg)/0.167 \times (0.033\ kg)/(0.055\ m^3)$$

$$ITSL = 7.79 \times 10^{-3}\ mg/m^3$$

$$ITSL = 8\ \mu g/m^3 \text{ (based on annual averaging time)}$$

In a developmental study, groups of 6 to 15 pregnant C3H/HeNJc1 mice were given doses in drinking water of 0, 3000, 6000, or 12000 ppm TETA on gestational day 0 to 19 (Tanaka et al., 1992). Maternal body and liver weight were determined. Maternal blood and maternal and fetal tissues were analyzed for copper concentration. Dams were analyzed for body weight, litter size, gross abnormalities, and fetal viability. Fetal cerebral weight was determined. Compared to controls maternal body weight was decreased at 12000 ppm ($a = 0.05$). Maternal liver weight was not decreased at any dose level. The total number of offspring per dam, including dead and live fetuses, did not differ among the four groups. The total resorption of the litter occurred in 0 out of 6 pregnant dams given 3000 ppm, in 1 of 11 dams given 6000 ppm (medium dose), and 7 of 15

pregnant dams given 12000 ppm (high dose). No statistical analysis was performed on this endpoint[1]. Cerebrum weight was decreased in the 6000 and 12000 ppm dose compared to control (a = 0.05). Abnormalities were frequently observed in cranium or brain, including massive hemorrhages and hematomas, delayed ossification in cranium, exencephaly, microcephaly, and hydrocephaly. These brain abnormalities in live fetus were as follows (number of fetuses with abnormality per number of fetuses observed): 1.3% (1/79) in the 0 ppm group, 6.3% (2/32) in the 3000 ppm group, 8.5% (5/59) in the 6000 ppm group, and 39.0% (16/41) in the 12000 ppm group. After statistical analysis (Fisher's Exact Test) the incidences of abnormalities in the mid (P = 0.046) and high dose (P < 0.001) were found to be significantly increased over control. Copper concentration in the fetal liver and cerebrum was decreased (a = 0.05) at all doses compared to controls. The authors stated that the 3000 ppm dose is approximately 500 mg/kg. The authors did not present the mid dose in terms of mg/kg nor did they present drinking water consumption data needed to calculate the dose in terms of mg/kg. The mid dose of 6000 ppm was estimated to be about 1000 mg/kg (2 x the low dose). This dose (1000 mg/kg) was determined to be the developmental LOAEL based on decreased cerebral weight and increased brain or cranium abnormalities in the fetuses. A NOAEL was identified as 3000 ppm (500 mg/kg/day). A developmental ITSL was derived based on the developmental reference dose (RfD_{DT}) (EPA, 1991).

$$RfD_{DT} = NOAEL/(UF_1 \times UF_2)$$

Where NOAEL is the no observable adverse effect level,
UF_1 is an uncertainty factor of 10 for animal to human extrapolation,
UF_2 is an uncertainty factor of 10 for human to sensitive human extrapolation,

$$RfD_{DT} = (500 \text{ mg/kg})/(10 \times 10)$$

$$RfD_{DT} = 5 \text{ mg/kg}$$

The ITSL was developed pursuant to Rule 232(1)(b) as follows:

$$ITSL = RfD \times 70kg/20m^3$$

$$ITSL = 5 \text{ mg/kg} \times 70kg/20m^3$$

$$ITSL = 17.5 \text{ mg/m}^3$$

Developmental ITSL = 17,500 $\mu g/m^3$ (based on 24 hour averaging time)

In another developmental study, groups of 5 to 9 pregnant Sprague-Dawley rats (190-200 g) were dosed with 0, 0.17%, 0.83% or 1.66% TETA from

[1] In the 6000 ppm dose a Fisher's Exact test was performed by AQD staff. The incidence of total resorptions (1 out of 11) was not found to be significant (P = 0.45).

gestation day 0 to day 21 (Keen et al., 1983). The dose in mg/kg was calculated from the average feed consumption found in the report and the initial weight of the female rat (~200 g). The doses then become 0, 105, 893, or 1717 mg/kg. All dams fed TETA regardless of dose, appeared healthy and normal throughout the experimental period. There were no fetal resorptions in the control group. Maternal weight gain was decreased compared to control at 0.83% (893 mg/kg, $p< 0.05$) and 1.66% (1717 mg/kg, $p< 0.01$), "despite similar food intake". In the low, medium and high dose groups the fetal resorption incidence was 5.8%, 8.7% and 18.8% respectively. No statistical analysis was presented by the author; however, a Fisher's Exact Test was performed by Air Quality Division staff (see attachment). The P value for the low dose group was found to be 0.070. This was deemed not to be significant. The dose of 893 mg/kg had a significantly increased incidence of fetal resorptions compared to the control group ($P = 0.015$). The average litter size was not affected by TETA at any dose level; however, the incidence of abnormal fetuses was 0, 25.6, and 100% in the low, medium ($P < 0.001$), and high ($P < 0.001$) dose groups, respectively, compared to 0% in the controls. Fetal weight and crown-rump length was significantly decreased at 1717 mg/kg compared to control ($p < 0.01$ and $p < 0.001$ respectively). The lowest observed adverse effect level (LOAEL) in the dams was 893 mg/kg based on decreased body weight gain, however, an ITSL was not developed using maternal effects because of the limited number of endpoints evaluated. A LOAEL in the fetuses was also 893 mg/kg based on increased incidence of abnormal fetuses and fetal resorptions. The low dose group (105 mg/kg) was designated a developmental NOAEL. A developmental ITSL was derived based on the developmental reference dose (RfD_{DT}) (EPA, 1991).

$$RfD = NOAEL/(UF_1 \times UF_2)$$

Where UF_1 is an uncertainty factor of 10 for animal to human extrapolation,
UF_2 is 10 for human to sensitive human extrapolation,

$$RfD = (105\ mg/kg)/(10 \times 10)$$

$$RfD = 1.05\ mg/kg$$

The ITSL is then developed according to Rule 232(1)(b) as follows:

$$ITSL = RfD \times 70kg/20m^3$$

$$ITSL = 1.05\ mg/kg \times 70kg/20m^3$$

$$ITSL = 3.675\ mg/m^3$$

Developmental ITSL = 3,675 $\mu g/m^3$ (based on 24 hour averaging time)

Several factors were considered in establishing a screening level for TETA including the comparison of effect levels in human and animal studies, the duration of the studies, and the quality of the data. In the 35-year-old

patient (Epstein et al.) a 2 day LOAEL of 1200 mg/day (~ 17 mg/kg/day) (acute rhabdomyolysis) was observed. In rodents, after 19 days of oral exposure, both maternal and fetal effects were observed at 1000 and 893 mg/kg (Tanaka et al. and Keen et al., respectively). The NOAELs for these studies were 500 and 105 mg/kg, respectively. Humans appear more sensitive to TETA than rodents, however, this conclusion is based on very limited data (a single clinical study involving 4 patients). Furthermore, the human patients were quite ill before treatment and no controls were used. For these reasons the ITSL was not based on the human study. In the rodent studies the only toxic endpoints analyzed in the dams were maternal weight gain (in both studies) and absolute liver weight (only in Tanaka et al.). It seems likely that the lowest observed adverse effect level would be lower in the rodent dams if clinical and histopathology examinations were performed. Due to the limited number of endpoints evaluated, and because adverse effects were observed in humans at levels roughly 6 times lower than rodents (NOAELs of 17 mg/kg vs. 105 mg/kg) it was deemed inappropriate to use either of the 19 day maternal studies for the derivation of an ITSL protective of long term adverse effects in humans. The only other data available for developing an ITSL protective of chronic effects is the rat oral LD50 study (see page 2 for calculation). This study was therefore selected as the basis for determining the ITSL. With regard to the developmental ITSL the Keen et al. study was chosen instead of the Tanaka et al. study because effects were seen at a lower dose level in the Keen et al. study. (see page 4 for calculation).

The Developmental ITSL for TETA is 3,675 $\mu g/m^3$ (based on 24 hour averaging time). The ITSL for chronic effects is 8 $\mu g/m^3$ (based on annual averaging time).

REFERENCES

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CALCULATION OF P VALUE USING FISHER'S EXACT TEST

Table used for calculating P based on incidence

	positive	negative	total
Group 1 (control)	A	B	A+B
Group 2 (dose)	C	D	C+D
	A+C	B+D	A+B+C+D

$$P = \frac{(A+B)!(C+D)!(A+C)!(B+D)!}{(A+B+C+D)!A!B!C!D!}$$

EXAMPLE 1:

LOW DOSE vs. CONTROL - INCIDENCE OF FETAL RESORPTIONS

	positive	negative	total
Group 1 (control)	0	73	73
Group 2 (dose = 105 mg/kg)	3	49	52
	3	122	125

$$P = \frac{(73!)(52!)(3!)(122!)}{(125!)(0!)(73!)(3!)(49!)} = \frac{2.1362763 \times 10^{377}}{3.0715014 \times 10^{378}} = 0.06955 \approx 0.07$$

EXAMPLE 2:

MID DOSE vs. CONTROL

	positive	negative	total
Group 1 (control)	0	73	73
Group 2 (dose = 893 mg/kg)	7	86	93
	7	159	166

$$P = \frac{(73!)(93!)(7!)(159!)}{(166!)(0!)(73!)(7!)(86!)} = \frac{(93!)(159!)}{(166!)(86!)} = \frac{3.4086642 \times 10^{426}}{2.181333 \times 10^{428}} = 0.0153 \approx 0.02$$

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MD:slb