MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

February 4, 2004

TO: File for diethylbenzene mixture (25340-17-4)

FROM: Marco Bianchi

SUBJECT: Initial Threshold Screening Level

The Initial Threshold Screening Level (ITSL) for diethylbenzene (DEB) mixture is 6 µg/m^3 based on an annual 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.

Diethylbenzene is a colorless liquid that usually occurs as a mixture of isomers, predominately the *meta* and *para* isomers, with a lesser amount of the *ortho* isomer; it mainly serves as an intermediate for the production of divinylbenzene. Diethylbenzene has been reported to have chromogenic properties in rats, and there are some data that support a link between chromogenicity of chemicals and the development of polyneuropathies (Ganaire 1989).

A detailed database search on DEB resulted in a number of toxicity summaries and a neuropathy study that could be reviewed to derive an ITSL. The collection of toxicity summaries was prepared by the American Chemistry Council for U.S. EPA's High Production Volume Chemical Challenge Program, and included: two oral LD_{50} studies, a subchronic inhalation study, a reproductive study, and a developmental toxicity/teratogenicity study. The neuropathy study investigated tail nerve motor/sensory conduction velocities, and the amplitude sensory action potential.

In the acute oral toxicity study, groups of five Sprague-Dawley male or female rats were orally dosed with diethylbenzene according to FIFRA/TSCA guidelines. The animals were observed for 14 days, and the LD_{50} value was estimated to be 2050 mg/kg (confidence range of 1770 to 2330 mg/kg). A variety of abnormal signs occurred on the day of dosing. Several animals exhibited hypoactivity, red nasal discharge, urinary staining, partially closed eyes, prostration, and decreased food consumption. Signs seen in a few animals (in most groups) included ataxia, tremors, clear nasal and oral discharges, wet rales soft stool rectal staining and abdominal griping. A few animals exhibited blue pigmentation and hypothermia on the day of dosing; by day 2 or 3, a majority of the survivors exhibited these signs. Postmortem examination of animals which were found dead revealed a variety of changes, primarily blue pigmentation of all/most soft tissues and or blue fluid in the gastrointestinal tract and urinary bladder. Other changes seen in most dead animals included irritant and/or corrosive changes in the stomach, intestine and urinary bladder.

In another rat oral LD_{50} study, groups of five Sprague-Dawley male or female rats were orally dosed with diethylbenzene according to FIFRA/TSCA guidelines. The animals were observed for 14 days. The estimated LD_{50} value was 6900 mg/kg for males and 4700 mg/kg for females, much higher than the previous LD_{50} study (LD_{50} = 2050 mg/kg). No explanation was given for these differences. Adverse effects appeared to be indicative of higher dose levels compared to the previous LD_{50} study. Treated animals displayed motor dysfunction, tremors, central nervous system depression, and coma. Green urine was also observed in all dose groups. Cyanosis was present in all dose groups except one female group treated with 3400 and 4300 mg/kg. This adverse effect is considered the same as the blue stained tissues in the previous LD_{50} study.

In the 3 month subchronic rat inhalation study, 10 Sprague-Dawley rats/sex/group were exposed to a mixture of diethylbenzene at 200, 600, and 1200 mg/m^3 6 hrs/day, 5 days/wk according to EPA guidelines. Mean analytical concentrations equaled 0, 190, 610, and 1400 mg/m^3. Results included decreased mean body weights in the high-exposure group throughout the study. There were no abnormal clinical observations considered to be treatment related, or ocular abnormalities attributed to the test material. Treatment-related changes in hematologic parameters included moderate decreases in total white cell and lymphocyte counts in the mid- and high-exposure level males. Abnormal sera color (blue or blue-gray) was observed in high-exposure level animals of both sexes. Treatment-related changes in serum chemistry parameters included decreases in ALT, AST, and CPK in high-exposure level females, and increases in potassium in high-level group and females from the mid- and high-exposure groups. An abnormal blue-gray color was observed in most tissues from all but one high-exposure animal. At the mid-exposure level, the same color was observed in brains of eight males and all females and in the urinary bladders of five females and one male. This abnormal color probably resulted from the presence of the parent chemical or a metabolite in these tissues. However, there were no other gross or microscopic changes, including reproductive tissues attributed to the test material. The study investigators concluded that repeated exposure to mixed diethylbenzene did not result in any target organ toxicity. The NOAEL was determined to be 190 mg/m^3.

In an oral developmental/teratogenicity rat study, 25 female Sprague-Dawley rats/group were dosed with a mixture of diethylbenzene at 20, 100, and 200 mg/kg, on days 6 through 15 of gestation for 7 days/wk according to EPA guidelines. Females were euthanized on gestation day 20. There was no treatment-related mortality or clinical signs of toxicity throughout the study. Mean maternal body weight gain and food consumption were statistically reduced at the 100 and 200 mg/kg/day groups, along with a greenish-blue discoloration of the amniotic sac in a dose related manner. Mean fetal body weight was statistically reduced at the 200 mg/kg/day level when compared to the control group. All other cesarean parameters were comparable among groups. No treatment-related malformations or developmental formation were observed. The study investigators concluded that oral gavage dosing with up to 200 mg/kg/day of mixed diethylbenzene did not produce a teratogenic response in rats. Maternal toxicity occurred at dosages that were lower than for developmental toxicity. The maternal toxicity NOAEL was determined to be 20 mg/kg/day, whereas the developmental toxicity NOAEL was determined to be 100 mg/kg/day.

The neuropathy study consisted of two experiments. The first one, *Experiment A,* was conducted to determine if the diethylbenzene mixture could cause sensorimotor neuropathies in rats, and the

second one, *Experiment B,* whether this was the responsibility of a particular isomer. In Experiment A, 12 male Sprague-Dawley rats/group (10 rats/control group) were dosed with 0, 500, or 750 mg/kg diethylbenzene mixture in olive oil by oral gavage. Electrophysical measurements were conducted every 2 weeks. In Experiment B, 12 male Sprague-Dawley rats/group (10 rats/control group) were dosed with 100 mg/kg 1,2-DEB, 4 days/week for 8 weeks, or 500 mg/kg 1,3-DEB or 1,4-DEB, 5 days/week for 8 weeks. Rats were subjected to neurophysiological measurements every week during the treatment period. The survivors were kept for observation and neurophysiological measurements during an 8-week recovery period. In both experiments, the rats were observed daily and weighed weekly. Results from Experiment A included a blue discoloration of the skin and urine as soon as the 3rd day of treatment with either 500 or 750 mg/kg DEB. A significant reduction in weight gain was observed from the first week of treatment in the group treated with 750 mg/kg. In the high dose group, two animals died during the first week of treatment; there were seven and four surviving rats at weeks 5 and 10, respectively. Two rats died in the low-dose group during the 4th and 7th weeks. No animals died in the control group. Rats in treated groups developed severe weakness in hind limbs and disturbance in gait from the 4th week of the experiment. This weakness got worse in the following weeks, resulting in a complete paralysis of the hind limbs for some rats. Results from Experiment B included the same symptoms (decreased body weight, blue discoloration of the skin and urine, weakness of hind limbs, paralysis) as those described above. One rat died in the first week of treatment and another in the 5th week of treatment. 1,3- and 1,4-DEB-treated rats did not display any signs of neurotoxicity or any other signs of systemic toxicity. However, 1,4-DEB-treated rats temporarily presented a body weight significantly lower than control rats from the first week to the fourth week of treatment. During the recovery period, the 1,2-DEB-treated rats regained weight, became more mobile but presented trailing hind limbs when attempting to walk. On the fourth week of recovery, all animals treated with 1,2-DEB succeeded in standing up.

Results from the neurological study indicates that 1,2-DEB (ortho isomer) is the metabolite that leads to the bluish discoloration in soft tissues, and appears to be the most toxic isomers. It is a strong irritant that results in systemic toxicity (gastrointestinal, urological, hematological, etc.) in addition to producing neuropathies. Because this compound appears to affect so many organ systems, it seems appropriate to use the toxicity study that would provide the most safety to sensitive subpopulations. Data from the 90-day inhalation study was of good quality to derive an ITSL but didn't consider maternal toxicity, which is the most sensitive endpoint. An ITSL derived from the teratogenicity study would result in a lower value than one derived from the 90-day inhalation study. Both studies resulted in test animals developing a blue discoloration suggesting that DEB mixtures had metabolized to 1,2-DEB and was systemically present in the animals. Therefore, the ITSL will be derived from the teratogenicity study that resulted in a NOAEL of 20 mg/kg

The ITSL was derived as follows:

NOAEL = 20 mg/kg
35 = uncertainty factor; to account for using a NOAEL for a 7-day exposure period to estimate a NOAEL for a lifetime study.
100 = uncertainty factor; to account for specie differences and human population sensitivities.

$$ITSL = \frac{NOAEL\ (mg/kg/day)}{35 \times 100} \times \frac{W_A}{I_A} \times \frac{b}{a}$$

$$ITSL = \frac{20\ mg/kg/day}{35 \times 100} \times \frac{0.338\ kg}{0.328\ m^3} \times \frac{1}{1}$$

$ITSL = 0.0059\ mg/m^3$

The ITSL for diethylbenzene mixture = 6 µg/m^3 based an annual averaging.

References:

1. Mercieca, MD. 1992. Teratology Study in Rats with MCS 2313 [mixed diethylbenzene]. Springborn Laboratories, Inc. Report No. 30344.228. Conducted for Monsanto Company.

2. Gagnaire, F. et al. 1990. Diethylbenzene-induced sensorimotor neuropathy in rats. Journal of Applied Toxicology 10(2), 105-112.