MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

September 8, 1999

TO: File for ethyl acetoacetate (141-97-9)

FROM: Marco Bianchi

SUBJECT: Initial Threshold Screening Level

The initial threshold screening level (ITSL) for ethyl acetoacetate is 46 $\mu g/m^3$ based on an annual averaging time.

The following references or databases were searched to identify data to determine the ITSL: IRIS-online, HEAST, NTP Management Status Report-online, RTECS, EPBCCD, EPB library, CAS-online, NLM-online, IARC-online, NIOSH Pocket Guide, and ACGIH Guide.

Ethyl acetoacetate is used as a flavoring agent for food and as a chemical intermediate for yellow pigment in paints, lacquers, inks and dyes. This compound was found to be weakly mutagenic in an Ames test with E. coli, but no mutagenic activity was detected when ethyl acetoacetate was tested with Salmonella typhimurium (in the presence of rat liver S-9 mix), and there was no evidence of chromosomal damage by this compound in Chinese hamster fibroblasts (Cook et al.).

In an LD_{50} study by Smyth et al, (1948), five rats were orally dosed in a geometric series with ethyl acetoacetate. Animals were observed for 14 days post-dosing. The LD_{50} was determined to be 3.98 g/kg. This value is comparable to a mouse LD_{50} of 5.1 g/kg listed in RTECS. Other short-term studies have shown this compound to be slightly to moderately irritating to the eyes and skin of rabbits.

In a sub-acute feeding study by Cook et al, (1992), 16 male and 16 female Sprague-Dawley rats were fed 100, 300, or 1,000 mg/kg ethyl acetoacetate encapsulated in gum arabic for 28 consecutive days. An additional group of 16 male and 16 female rats were given rodent diet containing gum arabic as a control. According to the investigators, the administration of ethyl acetoacetate in the diet did not adversely affect the growth or general health of the animals or their food intakes. They also stated that none of the minor variations observed in the hematology, serum chemical analyses or urine analyses were considered to

be indicative of a treatment-related toxic effect. Cecal enlargement was seen in male rats treated with the top dose of ethyl acetoacetate, but this was accompanied by a normal histopathology. Few histopathological abnormalities were observed. Proteinaceous casts were found in the bladder of approximately half the male rats in the 1000 mg/kg dose group. Renal function was unimpaired in treated male and female rats, and the histopathological findings was considered common in the strain of rats chosen for this study. Although the cecal enlargement and the changes in kidney and bladder of rats given 1000 mg ethyl acetoacetate/kg are noted, the investigators considered that ethyl acetoacetate did not produce treatment-related adverse effects in rats during this study.

The investigators concluded that in the absence of related concomitant structural and functional changes, none of the effects could be considered toxicologically significant. However, given the short study time, potential adverse effects could develop from the number of hematological parameters that were significantly different from controls. For example, red blood cell-mean cell volume was higher for all dose levels in males and all but the lowest dose level in females. Mean cell hemoglobin concentration, and hematocrit count was higher in the two highest dose groups in females. Additionally, platelets were also statistically higher in the two highest dose groups in males, compared to controls. White blood cell counts were lower in treated female rats than in controls, with a significant reduction in rats given 300 mg ethyl acetoacetate. Serum chemistry values were also significantly different in treated animals as compared to controls. Protein and albumin concentrations were significantly higher in female rats given 300 mg/kg and the albumin concentration was significantly higher than the control values for male rats in the high dose group. Sodium concentrations were significantly higher for all three treated groups of male rats compared with the control values. Cholesterol levels were higher than those of the control group in the three treated groups of male rats, and for the top dose the difference was statistically significant. In about half the male rats treated with 1000 mg/kg, statistically significant deposits of proteinaceous casts were present in the urinary bladder compared with only one such incident in the control group.
Clearly, administration of ethyl acetoacetate didn't cause one or two dose-related effects, but a number of dose-related effects affecting similar physiology.

According to the investigators, all of the dose-related effects were within historical ranges for the test specie even though most of these effects were statistically significant. It seems inappropriate to completely dismiss all of the dose-related effects in the study and state the highest dose as a NOAEL (no-observable-adverse-effect-level). Many of the changes occurred in all dose groups, or in both sexes, and mainly affected the hematological and serological systems. Instead, a NOAEL of 100 mg/kg (lowest dose) based on dose-related changes in red blood cell-mean cell volume and blood platelet changes that occurred in males will be used to derive an ITSL. These changes were not associated with pathogenic or organ weight changes.

NOAEL = 100 mg/kg/day.
Actual calculated intake of ethyl acetoacetate = 106 mg/kg/day
Uncertainty factor of 35 reduced to 25 because of 4 week (28 day) study

$I = 0.80(0.431)^{0.8206} = 0.401\ m^3$

$$ITSL = \frac{NOAEL}{25 \times 100} \times \frac{W_A}{I_A} \times \frac{b}{a}$$

$$ITSL = \frac{106\ mg/kg/day}{25 \times 100} \times \frac{0.431\ kg}{0.401\ m^3} \times \frac{1}{1} = 0.04557\ mg/m^3$$

Conversion of mg/m^3 to $\mu g/m^3$

$0.04557\ mg/m^3 \times 1000 = 45.6\ \mu g/m^3$

The ITSL for ethyl acetoacetate = 46 $\mu g/m^3$ based on annual averaging.

References:

1. Cook WM., et al. 1992. *A 28-day feeding study with ethyl acetoacetate in rats.* Fd.
Chem. Toxic. Vol. 30, No. 7, pp567-573.

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