MICHIGAN DEPARTMENT OF NATURAL RESOURCES

INTEROFFICE COMMUNICATION

March 14, 1995

TO: File for Tetrachloropicolinic Acid(CAS # 10469-09-7)

FROM: Dan O'Brien

SUBJECT: Initial Threshold Screening Level for Tetrachloropicolinic Acid (TCPA)

The initial threshold screening level (ITSL) for tetrachloropicolinic acid is 21 $\mu g/m^3$ based on an annual averaging time.

The following references or databases were searched to identify data to determine the ITSL: AQD chemical files, IRIS, HEAST, ACGIH TLV Booklet, NIOSH Pocket Guide to Chemical Hazards, RTECS, NTP Management Status Report, EPB Library, IARC Monographs, CAS On-line and NLM/Toxline (1967 - February 3, 1995), CESARS, Handbook of Environmental Data on Organic Chemicals, Patty's Industrial Hygiene and Toxicology, Merck Index and Condensed Chemical Dictionary.

The only data available concerning the use of tetrachloropicolinic acid was provided by the Dow Chemical Company (Gorzinski *et al.*, 1979; Norris, 1971). These sources indicate the chemical has been used as a starting material for the active ingredient (3,6-dichloropicolinic acid) in a herbicide, and as a processing intermediate for a sulfone to be used in latex paints.

Literature: Despite complete searches of the above references, only one toxicology report involving TCPA was located in the public literature, that of Rao *et al.* (1981). This study was a dermal sensitization trial of many classes of chemicals in which groups of ten randomly bred male Hartley strain guinea pigs (wt. $\cong$ 300 g) had 0.1 ml of the test agent applied to their clipped and depilated backs four separate times over a ten day period. A positive control group was utilized. At the time of the third application, 0.2 cc of Freund's adjuvant was injected intradermally at a single point adjacent to the application site. After two weeks, the animals were antigenically challenged on their clipped flanks (agent on one side; solvent, if used, on the other). The challenge sites were evaluated for erythema/edema at 24 and 48 hours post-challenge. Moderate erythema and/or edema in two or more animals was considered sufficient to classify an agent as a potential human sensitizer. Ten of the ten guinea pigs exposed to and subsequently challenged with TCPA showed skin sensitization, and consequently, TCPA was concluded to be a potential human sensitizer.

Unpublished acute range finding (Norris, 1971) and subacute (Gorzinski *et al.*, 1979) toxicity studies of TCPA were provided for review by Dow Chemical Company. In the acute study, groups of three mixed-sex, fasted, Sprague-Dawley rats weighing 193-222 g (μ = 211, σ = 8.5) received single doses of TCPA as a 10% suspension in corn oil by gavage at dose levels of 126, 252, 500, 1000 or 2000 mg/kg body weight. The animals were weighed prior to dosing, and at days 4, 8 and 15 post-dose. With the exception of the highest dose group, one male animal from each exposure group was decapitated and submitted for necropsy on day 4 post-dose; the remaining animals were

observed for fourteen days after dosing. One male in the 2000 mg/kg group died two days post-dosing, and was submitted for postmortem examination two days later (on day 4); this rat was the only animal examined in the 2000 mg/kg group. At necropsy, the only gross lesion reported for this rat was the presence of dark green fluid feces throughout the entire intestinal tract. Necropsied animals from all the other exposure groups showed no visible lesions. The remaining animals all survived and continued to gain weight to the termination of the study; no clinical signs were reported for any rat on study. No LD50 was calculated for this trial, but the 1000 mg/kg dose level may be considered the no observed adverse effect level (NOAEL) for this study, based on the lack of clinical signs or mortality, and the fact that weight gain appeared unaffected. Deficiencies of this study include the small number of animals per dose level, inadequate information regarding the purity of the test material and whether the animals were allowed an acclimation period prior to dosing. In addition, the total volume of vehicle plus test material did not remain constant for all of the dose levels.

The subacute study exposed six to seven week old male and female CDF Fischer 344 rats, 5 per sex per group, to TCPA incorporated into lab chow at concentrations of 0, 22, 66, 200 and 600 mg/kg/day for 31 days. The rats were observed at least twice weekly for clinical signs; body weight and food consumption were recorded weekly. Clinical chemistry (blood urea nitrogen (BUN), alkaline phosphatase (SAP) and alanine transaminase (SGPT)) were determined on blood samples obtained at termination; hematology (packed cell volume, erythrocyte count, hemoglobin concentration, leukocyte count, and leukocyte differential) was performed on day 25 for control and top dose animals. Urinalysis was carried out on day 25 for animals in the control and 600 mg/kg/day groups. All rats were necropsied following decapitation at the termination of the study. Each was examined for gross lesions, and brain, heart, liver, kidney and testicular weights obtained. Representative samples of all major organs were obtained and fixed for histopathological examination in the control and high dose groups; sections of liver and kidney only were prepared for rats in the 22, 66 and 200 mg/kg/day groups. Measured parameters were evaluated for statistical significance using one way analysis of variance, with comparisons between dose groups carried out via Dunnett's test.

There was no mortality, nor any overt clinical signs recorded in any of the animals. There were no statistically significant differences from controls in mean body weights at any point in the study. The feed consumption of the high dose females was significantly ($p < 0.05$) decreased compared to the control females during the first three days of the study, but consumption rebounded thereafter and subsequent feed consumption was not significantly different from controls in any dose group of either sex. The early decrease in the high dose females was attributed to adaptation to the test diet. Of the clinical pathology tests carried out, the only statistically significant deviations from control values were: 1) a decrease in mean BUN for the high dose males, 2) decreased SAP in the 200 mg/kg/day females and 3) increased urine specific gravity in high dose males. Of these, only 1) was considered an exposure-related effect. The depressed SAP did not occur in any of the males or any of the other female dose groups, and was considered an artifact. The deviation in urine specific gravity in the high dose males led the researchers to repeat the urinalysis, this time incorporating all of the dose groups, the next day. None of the specific gravity measurements at that point were significantly different from controls. At necropsy, no exposure-related gross lesions were noted in any of the animals at any dose. Histopathologic lesions considered to be related to treatment were confined to the liver and present only at the 200 and 600 mg/kg/day dose levels. At the highest dose level, "very slight to slight" centrilobular hepatocellular swelling and decreased staining intensity were present in all five animals of both sexes; at the 200 mg/kg/day level, these lesions were exhibited by all five males, but

none of the females. There were no hepatic lesions related to exposure at any of the other dose levels.

Methodological Issues: Due to its length of exposure, number of dose groups, and multiple measured endpoints, the 31-day feeding study is clearly the best available study for use in the derivation of a screening level. However, some methodological difficulties in the study must be addressed. Aspects of the statistical analyses conducted in this study are suspect. At the end of the study, the authors excluded one of the male control animals from analysis on the grounds that it constituted "a statistical outlier". The discussion states "this was effected to facilitate interpretation of the results without the existence of the aberant (*sic*) control value (the final body weight of the animal eliminated was approximately 130% of the mean weight for the remaining control male rats)". However, the excluded animal's final body weight, when compared with the mean body weight of all the other males on trial, was somewhat less (122%). No details are provided as to the statistical test which was performed (if any) to identify this animal as an outlier, leading one to wonder if exclusion of this animal was purely subjective. While there is no question that this animal was the largest animal at the end of the study, it was the largest animal at the beginning of the study as well, twenty grams heavier than the next heaviest rat. If the animal was to be excluded based on body weight, the appropriate time to do so would have been at initiation of the study. A description of the method of assignment to dose groups is also conspicuously absent from this study. This, coupled with the fact that two of the three lightest animals were assigned to the control group along with the heaviest rat, leaves open the possibility that group assignment was non-random. One can only speculate that these group assignments may have been employed to make the between-group differences in mean body weight statistically insignificant.

The statistical techniques employed to compare differences in measured parameters (one way analysis of variance, followed by Dunnett's multiple comparison test) are appropriate for the study. However, Dunnett's test is not invariant to different sample sizes in the groups it compares (Zar, 1974; Day and Quinn, 1989). Here, no indication was given by the investigators as to whether the different dose group sizes resulting from the exclusion of the "outlier" were accounted for in the analysis, as they must be to yield valid results. Consequently, the raw data for absolute and relative liver and kidney weights were re-analyzed using the SAS System, Version 6.08 for Windows (Proc GLM, SAS Institute, 1989). The statistical method employed for re-analysis was identical to that specified by the study authors, with the exception that a modification of Dunnett's test which controlled for differing dose group sizes was used (A copy of the SAS code used and the results obtained are included in the file for this chemical).

The statistical significance of the critical effect (liver weight changes) upon which the NOAEL is based was effected by the deletion of the "outlier"; this is why the statistical deficiencies of this study are relevant. With respect to liver weight changes, results of our re-analysis agreed with the investigator's findings only with the "outlier" removed prior to analysis, but not with the animal included. Our data entry and re-analysis were checked for errors that might have accounted for the disparate results, but none were identified. Given the noted problems with the statistical analysis in this study, and the fact that deletion of the "outlier" is in our opinion inappropriate, the results of our re-analysis, with *all* the male animals included, were considered most likely to be reliable; these were used to determine the NOAEL, and to drive the derivation of an ITSL.

NOAEL Determination: Absolute liver weights of the 600 mg/kg/day male rats were significantly increased ($p \leq 0.05$) compared to controls, and relative (g/100 g body weight) liver weights

were significantly increased with respect to controls in all exposed groups of male rats. Among the females, there was a significant increase from control values for relative liver weight in the highest dose group. Relative kidney weights were significantly increased relative to controls at the two highest dose levels in male rats, while in females, only the 600 mg/kg/day group showed a significant increase over controls. No other organ weights were significantly different from control in either sex at any dose level by the investigators' analysis with the "outlier" included. The significant increase in relative liver weight at the 22 mg/kg/day exposure level is the lowest dose in this study at which an exposure-related effect was noted. While the 22 and 66 mg/kg/day dose levels noted significant changes in relative liver weights, there was no accompanying increase in SGPT or SAP, nor any exposure-related histopathological lesions, at either dose level that would indicate the presence of a pathological process associated with this increase in relative weight. Moreover, accompanying deviations in the weights of other organs are not present at either the 22 or 66 mg/kg/day level. Consequently, while the significant increases in relative liver weights in these two dose groups are clearly effects, other study data do not support the interpretation that they are adverse effects. At the 200 mg/kg/day level in males, both relative liver and relative kidney weights are significantly increased relative to control values; histopathologic hepatocellular lesions related to exposure, though slight, are present as well. This is considered sufficient evidence that the effects of TCPA exposure at the 200 mg/kg/day dose level are adverse in male rats. Thus, 200 mg/kg/day is considered the lowest observed adverse effect (LOAEL) in this study, and the next lowest dose level, 66 mg/kg/day, is considered the NOAEL.

ITSL Derivation: Applying Rule 232(1)(e) of Act 348,

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

where:

W_A = Mean final (fasted) body weight of male F344 rats in the key study
I_A = Daily inhalation rate of a male F344 rat (default value from MDNR, 1991)
b = Absorption efficiency by the oral route of exposure
a = Absorption efficiency by the inhalation route of exposure

So,

$$\textbf{ITSL} = \frac{66 \text{ mg/kg/day}}{3500} \times \frac{0.268 \text{ kg}}{(0.925 \text{ m3/kg/day} \times 0.268 \text{ kg})} \times \frac{1}{1}$$

$$= 0.019 \text{ mg/kg/day} \times (0.925 \text{ m}^3\text{/kg/day})^{-1} \times 1$$

$$= 0.021 \text{ mg/m}^3 \times \frac{1000 \text{ µg}}{1 \text{ mg}}$$

$$= \textbf{21 µg/m}^3$$

with (b/a) taking on the default value of 1 in the absence of data to the contrary.

Per 232(2)(c), an **annual averaging** time applies.

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