MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

September 13, 1999

TO: File for Diallyl phthalate (131-17-9)

FROM: Marco Bianchi

SUBJECT: Initial Risk Screening Level

The initial risk screening level (IRSL) for diallyl phthalate is 0.1 $\mu g/m^3$ based on an annual averaging time.

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

A complete literature search was conducted for diallyl phthalate, but information could only be obtained from two National Toxicology Programs (NTP) companion bioassays for this chemical evaluation. The first bioassay was a carcinogenic gavage study conducted on B6C3F1 mice. This study was published in April 1983. The second bioassay was a toxicology and carcinogenic gavage bioassay conducted on F344/N rats, and was suppose to run concurrently with the mouse study. But, due to a dosing error, this study had to be restarted and was subsequently published in August 1985.

Diallyl phthalate is a widely used crosslinking agent for unsaturated polyesters. This compound and similar chemical blends are used primarily as plasticizers and carriers for adding catalysts and pigments to polyesters and in molding, electrical parts, laminating compounds, and impregnation of metal castings.

According to NTP, the pharmacokinetics of diallyl phthalate have not been studied extensively, but other dialkyl phthalate esters appeared to be easily hydrolyzed to their corresponding alcohols and monoalkyl phthalates possibly in the gut prior to intestinal absorption. Consequently, the parent compound was not isolated from the tissues of rats 4 hrs after oral administration of near-lethal amounts of diallyl phthalate. The proposed metabolism of diallyl phthalate has been demonstrated by the isolation of a single metabolite, 3-hydroxyproyl-mercapturic acid, in the urine of rats administered this compound. Since allyl

alcohol and acrolein are also excreted as 3-hydroxypropyl-mercapturic acid in the urine of rats, it has been hypothesized that one or more ester linkages of diallyl phthalate are initially hydrolyzed and that the released allyl alcohol is then oxidized to acrolein. Acrolein reacts with glutathione and is then reduced to an alcohol and excreted as the N-acetylcysteine conjugate. The conjugation of acrolein with glutathione occurs in the liver in vivo, but has not been demonstrated in other tissues. The major toxic effect being periportal hepatocellular necrosis due to the metabolites, allyl alcohol and acrolein. However, the hepatotoxic effects of allyl alcohol in rats regress despite continued administration, suggesting adaptation of the liver to the presence of allyl alcohol or acrolein. The mechanism of the developed resistance to allyl alcohol is not known.

Oral LD_{50} studies of diallyl phthalate have shown moderate toxicity to rats with values ranging from 0.77 to 1.7 g/kg. Single dose, oral LD_{50} studies were not found for mice during the literature review. In the 14-day NTP studies, 5 mice/sex/group and 5 rats/sex/group were administered 0, 50, 100, 200, 400 or 600mg/kg of diallyl phthalate in corn oil. For the mice, there were no differences in mean body weight from controls and no clinically related lesions observed at necropsy. However, 50% of the mice died at 400mg/kg. Comparatively, in rats, deaths occurred at 600 mg/kg (all rats from this dose group), and 3/5 males and 1/5 females at the 400 mg/kg dose group. At necropsy, dark, mottled lungs and distended stomachs were observed in both males and females at 400 and 600 mg/kg, and for all but one animal in the 200 mg/kg dose group. Abnormalities in the appearance of the liver were observed in all animals from all dose groups, but in differing degrees of severity. At 200-600 mg/kg, the livers appeared to be enlarged, dark, and mottled, but at 50 and 100 mg/kg, the mottling was less severe and the enlargement of the liver not grossly apparent.

In the 13-week NTP studies, 10 male and 10 female, mice and rats/group were dosed at 0, 25, 50, 100, 200, and 400 mg/kg. Results from the mouse study revealed only a single death in male mice at the 400 mg/kg group. Likewise, only one death occurred in each of the dose groups in 0, 25, 50, 200, and 400 mg/kg for female mice. Three of these six deaths were unequivocally caused by accidents; the other three animals did not exhibit pathologic lesions that were clearly compound related. None of the deaths were considered to be chemically induced. Additionally, neither gross nor microscopic alterations related to chemical administration were observed in any of the high-dose mice. In contrast, the rat study resulted in 6/10 male rats dying during the study that received 400 mg/kg, and another two were killed when found in a moribund condition. Initial feed consumption was down with a corresponding depression in body weight for the 400 mg/kg dose group. Clinical signs of toxicity were observed in males and females at the 400 mg/kg throughout the studies, and less frequent in both sexes at 200 mg/kg. The clinical signs consisted of diarrhea, rough hair coat or alopecia around the head, hunched posture, and general emaciation. No clinical signs were observed in lower dose groups. At necropsy, gross abnormalities of

the liver were observed in all eight 400 mg/kg male rats that died early. The livers appeared to be enlarged, mottled (mainly yellow blotches) and pale; the surface texture was rough granular, or pitted. The lungs in many of these males appeared darkened or bright red. The severity appeared to be dose related in males and greater in males than in females. The kidneys of female rats at 400 mg/kg were considered to have abnormal (greenish-brown) coloration. Histologic examination indicated that the liver was the primary target organ. The lesions occurred principally in the periportal regions of the hepatic lobules. Periportal hepatocellular necrosis and fibrosis, bile duct hyperplasia, and hepatocellular nodular hyperplasia occurred in other males and females at 200 and 400 mg/kg. Necrosis, fibrosis, and biliary hyperplasia were not observed at doses lower than 200 mg/kg, but hepatocellular alterations in the periportal region were observed with decreasing frequency and severity at doses as low as 50 mg/kg (males) or 100 mg/kg (females). The cellular alterations were characterized by hepatocellular basophilia, cellular and nuclear hypertrophy, and nuclear hyperchromatism. The severity of the hepatocellular alterations was subjectively graded as moderate to severe in both sexes at 200 mg/kg and mild at lower doses.

In the carcinogenesis bioassay a dose of 0, 150, or 300 mg/kg diallyl phthalate in corn oil was administered by gavage to groups of 50 male and 50 female B6C3F1 mice, 5 days/ week for 103 weeks. Survival rates and mean body weights of dosed mice were not different from those of controls, and pathological lesions unrelated to proliferative changes were not observed. Therefore, a maximally tolerated dose for the purposes of carcinogenicity testing may not have been achieved. The incidences of lymphoma and either lymphoma or leukemia in dosed male mice were not significantly greater than those in the controls according to pairwise comparisons, but the trend tests were statistically significant by either life table or incidental tumor analyses. The incidence of lymphomas in the high-dose male mice was 12/50 in comparison with 6/50 in the controls. Since the incidence of high-dose male mice with leukemia was not significantly greater than that of concurrent or historical controls by pairwise comparisons, this marginal increase was considered only to be equivocally related to diallyl phthalate administration. Increased incidences of squamous cell papillomas, hyperplasia, and inflammatory lesions of the forestomach were observed in treated mice of both sexes in a dose-related manner. Papillomas of the forestomach were observed in 0%, 2% and 4% of the control, low-dose, and high-dose mice of both sexes. Historical incidence of this tumor type in gavage control mice was less than 1%. Forestomach hyperplasia was diagnosed in 0%, 15%, and 18%, of the control, low-dose, and high-dose males and in 8%, 2%, 29% of the control, low-dose, and high-dose female mice. Because of the numerical elevation of forestomach papillomas in high-dose mice of both sexes, the concomitant observation of dose-related forestomach hyperplasia, and the rarity of this tumor in corn oil (gavage) control B6C3F1 mice, development of squamous papillomas of the forestomach may have been related to diallyl phthalate administration. According to the NTP, under the conditions of the

bioassay, the development of chronic inflammation and hyperplasia of the forestomach in both male and female B6C3F1 mice was considered to be related to the administration diallyl phthalate. The development of squamous cell papillomas of the forestomach may also have been related to chemical administration, but the available data are insufficient to indicate a clear cause and effect relationship. An increase in the incidence of male mice with lymphomas was observed, but this increase was considered only to be equivocally related to diallyl phthalate administration. Therefore, say the investigators, results of this bioassay do not indicate that diallyl phthalate is carcinogenic in B6C3F1 mice, although a maximal tolerated dose may not have been achieved.

In comparison, diallyl phthalate in corn oil was administered by gavage to groups of 50 male and 50 female F344/N rats at doses of 0, 50, 100 mg/kg 5 days/week for 103 weeks. Mean body weights and survival of male and female rats administered diallyl phthalate were essentially the same as those of the vehicle controls throughout the 2-year studies, although hepatotoxicity was produced in both sexes at the 100 mg/kg dose. Hepatotoxicity was characterized by periportal fibrosis, periportal accumulation of pigment, and severe bile duct hyperplasia. Pigment accumulation also occurred at the 50 mg/kg dose in both sexes. Diallyl phthalate administration increased the occurrence of mononuclear cell leukemia in female rats and the incidence in the 100 mg/kg dose female rats was greater than in the vehicle controls by pairwise comparisons (vehicle control, 15/50, 30%; low dose, 15/43, 35%; high dose, 25/49, 51%). An increased occurrence of mononuclear cell leukemia was not observed in male rats receiving diallyl phthalate. According to NTP, under the conditions of this study, the administration of diallyl phthalate by gavage in corn oil to male and female F344/N rats for 2 years caused chronic liver disease characterized by periportal fibrosis and pigment accumulation and an increased severity of bile duct hyperplasia. The incidence of mononuclear cell leukemia was significantly increased in female rats receiving 100 mg/kg. Because of the variability in the incidence of this neoplasm in aged Fischer 344 rats and the difficulty in definitively diagnosing this lesion in Fischer 344 rats, this increase was considered to be equivocal evidence of carcinogenicity of diallyl phthalate in female rats. There was no evidence of carcinogenicity in male rats.

Other compounds containing the allyl group appear to be associated with malignancies of the hematopoietic system in rodents. Alylisovalerate and allylisothiocyanate increased the occurrences of mononuclear cell leukemia in male rats, and the former increased the occurrence of malignant lymphomas in female mice. In its deliberations, the NTP considered arguments both supporting and detracting from the significance of the observed increase in mononuclear cell leukemia in female rats as evidence of a carcinogenic response. The NTP debated the respective merits of all of the arguments and came to the consensus that, although none of the designated categories of evidence clearly described the diallyl phthalate results, the data could arguably

be supported in either the categories as *some evidence* or *equivocal evidence*. Following a lengthy discussion, however, the consensus of the Panel was that *equivocal evidence* of carcinogenicity was the most appropriate category of evidence due to the nondefinitive nature of the diagnosis of mononuclear cell leukemia in aged F344 rats. Their supporting arguments were as follows:

- The apparent increase in mononuclear cell leukemia was statistically significant by both trend tests and pairwise comparisons. Moreover, the strongest statistical evidence of an effect was provided by the survival-adjusted life table test, the analysis most appropriate for a lethal neoplasm such as mononuclear cell leukemia. The results of this test suggest that mononuclear cell leukemia occurred earlier in the higher dose female rats than in the vehicle controls.
- The occurrence of mononuclear cell leukemia in vehicle control female rats in this study was within the range observed for historical controls at the performing laboratory and somewhat greater than the historical control rate program-wide. Thus, the apparent increase in mononuclear cell leukemia in the higher dose animals was not a result of an abnormally low rate in the controls.
- Although the historical incidence of mononuclear cell leukemia in control female rats appears to be highly variable, the overall rate observed for the higher dose female rats in this study, 25/49 or 51%, exceeded the highest overall rate ever observed in control female rats in this program, 21/50 or 42%.

The data from incidence rates of leukemia in the female rat provided the highest unit risk value, and therefore, consistent with Rule 231(3)(b) was used to analyze ambient impacts of diallylphthalate.

The unit risk value was determined as follows:

MLE dose on $1x10^{-6}$ risk = 0.1169867751
95% Upper Confidence Interval = 9.461060 E-4

$$q_1^* = \frac{9.461060\ E\text{-}4}{0.1169867751} = (8.1x10^{-3}\ mg/kg/day)^{-1}\ (animal)$$

animal to human conversion:

$$q1^*(human) = q1^*(animal) \times \sqrt[3]{70kg / w(kg)}$$

weight of female rats averaged from groups = 250 grams

$$q1^*\ (human) = 8.1x10^{-3}\ (mg/kg/day)^{-1} \times \sqrt[3]{70kg / 0.250kg} = 0.053(mg/kg/day)^{-1}$$

oral to inhalation dose:

$$0.053(mg/kg/day)^{-1} \times \frac{20m^3}{70kg} \times \frac{1mg}{1000\ ug} = 1.5x10^{-5}\ (ug/m^3)^{-1}$$

Utilizing the unit risk value incidence rate of leukemia in the female rat, an ambient air concentration corresponding to an increased cancer risk of one in a million ($1x10^{-6}$) was determined as follows:

$$\text{Concentration} = \frac{1x10^{-6}}{1.5x10^{-5}\ (ug/m^3)^{-1}} = 0.06ug/m^3 \text{ or } 0.1ug/m^3$$

The IRSL for diallylphthalate = 0.1 ug/m^3 based on an annual averaging.

References:

1. U.S. Department of Health and Human Services. 1983. Carcinogenesis Bioassay of Diallyl Phthalate (CAS No. 131-17-9) in B6C3F1 Mice (Gavage Study). Carcinogenesis Testing Program; National Cancer Institute; National Toxicology Program. NIH Publication No. 83-1798. [NTP-81-83; TR-242].

2. U.S. Department of Health and Human Services. 1983. Toxicology and Carcinogenesis Studies of Diallyl Phthalate (CAS No. 131-17-9) in F344/N Rats (Gavage Studies). Carcinogenesis Testing Program; National Cancer Institute; National Toxicology Program. NIH Publication No. 85-2540. [NTP-81-83; TR-284].

MB:ST