MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

April 14, 2004

TO: File for poly(1,2-dihydro-2,2,4-trimethylquinoline) (26780-96-1)

FROM: Marco Bianchi

SUBJECT: Initial Threshold Screening Level

The Initial Threshold Screening Level (ITSL) for poly(1,2-dihydro-2,2,4-trimethylquinoline), also known as Flectol, is 35 $\mu g/m^3$ based on a 24-hr 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.

Flectol is an anti-oxidant used in the rubber manufacturing industry. According to staff at BF Goodrich (James Tanzilli, e-mail communication), Flectol is a polymer made up of a number of components of lower molecular weight oligomers of various structures. Unlike many high molecular weight polymers, this compound does not show a bell shape distribution of components and an average molecular weight. The average molecular weight *range* of Flectol is between 400-500.

Acute information for this compound is questionable. A Toxic Substance Control Act (TSCA) 8(e) submittal from Monsanto listed an LD_{50} value of 233 mg/kg for what is assumed to be Flectol, but this data sheet was sanitized of the chemical name. No other acute information could be found from the literature search.

A number of subchronic and chronic studies were also obtained through TSCA 8(e) submittals. Most of these submittals were rat dietary studies conducted by different manufacturers trying to verify the results of an initial 2-year dietary study by Panner and Packer (1961), showing liver toxicity and carcinogenicity.

In the Panner and Packer study, three separate tests (Parts A, B, and C) were conducted to determine the toxicity of Flectol. Parts A and B were short-term tests designed to determine toxicological endpoints. In Part C, 35 male and 35 female Rochester Wistar rats were maintained for 2-years on the following diets: control, 0.01%, 0.1%, and 1.5% (0, 20.5, 205, 3075 mg/kg, respectively) of Flectol. Only three males and 5 females given the 1.5% diet survived to sacrifice. According to the study investigators, gross

observations showed enlarged livers in the 1.5% group (male and female); female rats of the 0.1% diet group may also have had some liver enlargement. Three of the 5 female rats in the 1.5% diet group had one to several gross nodules in their livers. Where gross nodules were not present, whitish areas of less than one millimeter in diameter were seen in the livers of the rats on a 1.5% Flectol diet. Vascular channels were frequently dilated. No cirrhosis was seen. One rat of the 0.1% diet group had numerous nodules of metastatic tumor in the mesentery arising from the pancreas. Microscopic observations revealed the most marked change in the 1.5% diet group of rats. Gross nodules corresponded to the most atypical biliary-duct hyperplasia. Lumens contained mucin, eosinophilic debris, and leukocytes. Frequently, stratification of nuclei and mitoses were seen in the most atypical acini. The rest of the liver revealed smaller foci of duct hyperplasia, variation in hepatic cell size, and enlarged hepatic cells with eosinophilic cytoplasm. In summary, Flectol when fed to rats produced fatty change frequently localized to the midzonal area of the hepatic lobule. Chronic administration led to alteration of the hepatic cells, necrosis, and atypical biliary-duct hyperplasia. Although there were no listed adverse effects at 100 ppm (20.5 mg/kg), a no-observable-adverse-effect-level (NOAEL) was not established for this study.

As a follow-up to the Panner and Packer study, Monsanto and the B.F. Goodrich Company conducted 2-year rat dietary bioassays. It appears that each company wanted to clarify the results of the Panner and Packer study regarding any treatment-related effects at the 0.1% Flectol level. Of the two studies, the Monsanto bioassay was of better quality than the B.F. Goodrich study for the following reasons:

Monsanto	**B.F. Goodrich**
Conducted their own in-house testing. The report appeared to be very complete; with reviews of hematology, clinical chemistry ophthalmology, gross and microscopic pathology. All animals had individual examinations and recordings of clinical signs.	Contracted testing to outside laboratory; according to TSCA 8(e) correspondence between B.F. Goodrich and contract laboratory, B.F. Goodrich had concerns about the quality of the report. These concerns included: • No gross and microscopic pathology tables individually listing per animal; • No summary of total number of tumors; • No summary of pathology excluding tumors; • No summary of tumor bearing animals; • No individual listing per animal of recorded daily observations; and • All animals were recalled to be re-evaluated pathologically.
Four dose groups (0, 50, 250, 1000 ppm) to 60 rats/sex.	Four dose groups (0, 0.01, 0.1 and 1.5%) to 30 male and 20 female rats; however, highest dose group had complete mortality by the 10th week of study. Started a new control and high dose group of 0.5% Flectol at week 37.
Dosed an additional 15 rats/sex to evaluate hepatic cellular proliferation; sacrifices at 4-days, 1-month, and 6-months.	-

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Monsanto	B.F. Goodrich
ppm converted to mg/kg consumed.	Dietary doses expressed as % dietary level. Percentage dose converted by AQD to ppm, then mg/kg using EPA's default animal biological values.
Established a NOEL for non-carcinogenic effects, and a NOEL for carcinogenic effects. Effects seen at 250 and 1000 ppm.	According to report, hepatic changes including necrosis and biliary duct hyperplasia were observed, but the frequency of occurrence of these changes among the control and treated animals were similar. No NOAEL was established for this study. Explained away hepatic effects seen at 1000 and 5000 ppm.

Monsanto Study

This abstract includes interpretations and conclusions presented by the study investigators. In this study, Flectol was administered to groups of 60 Sprague-Dawley rats/sex at target levels of 0, 50, 250, and 1000 ppm in the feed for two years. An additional 15/animals/level/sex were used to evaluate hepatic cellular proliferation after four days on treated diet and at months 1 and 6. Clinical observations were performed weekly. Body weights and food consumption were determined weekly for the first thirteen weeks and every forth week thereafter. Clinicopathologic examinations were performed at 3, 6, 12, 18, and at termination (24 months). Ophthalmic examinations were performed pretest and just prior to termination. Ten animals/level/sex were sacrificed at month 12, and the remaining survivors were sacrificed at termination. All animals were given a complete necropsy. All retained tissues from the control and high level animals were examined microscopically. Liver, lung, spleen, gross lesions with possible histopathological correlates, and organs identified microscopically as potential targets at the high level were examined at the mid and low levels.

Analyses to verify the stability of the test material (both neat and when mixed in the diet), the dietary homogeneity, and concentrations of the test material in the diet were performed with satisfactory results. Overall study averages for consumption of test material (mg Flectol/kg/body weight/day) based on the target concentrations, were approximately 2.3, 11.8, and 48 in males and 3.1, 15.3, and 63 in females for the low, mid and high levels, respectively.

Decreased mean body weights in high level females throughout most of the study were indicative of toxicity. Slightly increased gamma glutamyl transpeptidase levels and heavier livers occurred in the high level of animals of both sexes. Relative to brain weight, the livers of mid level females were also heavier. Increased incidences of centrilobular hepatocellular vacuolation in high level females and of hepatic sinus dilatation in mid and high level females were also considered effects of compound administration. The hepatocellular vacuolation probably represented a minimal toxic effect on the hepatocytes. An increased incidence of thyroid follicular adenomas/cystadenomas in high level males and females was considered to have resulted

from compound administration, but may have resulted from compensatory mechanisms as a result of the hepatic changes.

Slightly increased incidences of hepatic foci of cellular alteration and of bile duct proliferation/cholangiofibrosis in high level males may have been related to compound administration. These spontaneous lesions are commonly observed in aged male rats. It is possible that the increased incidences of these lesions are commonly observed in aged male rats. It is possible that the increased incidences of these lesions in this group simply reflected more animals being at risk for a longer period of time, i.e., as a result of the higher survival rate in this group.

Slightly increased incidences of adrenal cortical cystic degeneration and of sinusoidal ectasia/cyst formation in high level males appeared to have been related to compound administration. However, these lesions are often observed in aged male rats and, in this study, were predominately observed in animals sacrificed at the end of the study. Based on these conclusions, the No-Observable-Effect-Level (NOEL) for toxicity was considered to be 11.8 mg/kg in males and 3.1 mg/kg in females. Target organs were liver and adrenal glands in males and liver in females. As a result of observing benign thyroid follicular adenomas/cystadenomas at the high level, the NOEL for oncogenicity was considered to be 11.8 and 15.3 mg/kg in male and females, respectively.

Supporting Studies

Of the remaining studies that were evaluated; one study (Hodge, 1966) reviewed the Panner and Packer investigation, while another study (British Industrial Biological Research Assoc.) was a six-week analysis sponsored by Monsanto that fed rats a diet containing 2,000 to 40,000 ppm of Flectol. All dietary levels except the 2,000 ppm level were halted after 20 days due to mortality and condition of the test animals. At the 2,000 ppm dietary level, adverse effects were similar to the Monsanto bioassay – increased liver weight, with liver vacuolation and enlargement around the portal tracts. The remaining study that was evaluated was a Monsanto teratology TSCA 8(e) submittal. This submittal provided preliminary results of rats dosed at 0, 20, 120, and 300 mg/kg/day (method unknown). Dams were treated for 10 consecutive days (gestation days 6-15). The highest dosage level tested, 300 mg/kg/day, also produced maternal toxicity which included significant increases in liver weights with accompanying decreases in body weight gain. While there were no decreases in the number of viable fetuses or a decrease in fetal weight, nor an increase in mean number of resorptions per dam, developmental toxicity was expressed. Developmental effects noted in the 300 mg/kg /day were kinked tail and or vertebra anomalies with or without associated rib anomalies. There was also an increase in some skeletal variances. The preliminary results indicated that the 20 mg/kg/day group was a NOEL for both maternal and fetal toxicity. The 120 mg/kg/day group did not produce any fetal toxicity and was considered the NOEL for developmental toxicity.

Data from the Monsanto dietary bioassay was sufficient to justify developing an ITSL using the oral reference dose (RfD) methodology according to Rule 232(1)(b). The report

appeared to be very complete; with reviews of body and organ weights, hematology, clinical chemistry, ophthalmology, gross and microscopic pathology. All animals had individual examinations and recordings of clinical signs, including types tumors and incidences of total and malignant tumors. Coupled with the teratology study and the other dietary studies, a comprehensive picture of Flectol dietary toxicity is obtained through this route of exposure. What is still uncertain; however, is the kind of toxicity that would be expressed if animals were exposed to airborne concentrations of Flectol. No information was found in the literature that would provide details of Flectol inhalation toxicity. Also, due to the inexact nature of the compound's molecular weight, it is uncertain if a 400 MW polymer would exert different biological effects compared to a 500 MW polymer. Therefore, in addition to the customary use of uncertainty factors, an added uncertainty factor (UF) of 3 will be used to account for the uncertainties presented above to adequately address all potential endpoints at various critical life stages of the animal. According to EPA, assuming the range of the UF is distributed log-normally, reduction of a standard 10-fold uncertainty factor by half results in a UF of approximately 3.

The ITSL was determined as follows:
NOEL = 50 ppm or 3.1 mg/kg for female rats (Monsanto study)

Uncertainty Factors

10 - specie to specie
10 - sensitive sub-populations
3 - uncertainty factor to account for database gaps

$$\frac{3.1 \text{ mg/kg}}{10 \times 10 \times 3} \times 0.010 \text{ mg/kg}$$

Conversion from *mg/kg* to *ug/kg*

$$0.010 \text{ mg/kg} \times \frac{1000 \text{ ug}}{1 \text{ mg}} = 10 \text{ ug/kg}$$

Conversion from *ug/kg* to *ug/m*^{3}

$$10 \text{ ug/kg} \times \frac{70 \text{ kg}}{20 \text{ m}^3} = 35 \text{ ug/m}^3$$

The ITSL for poly(1,2-dihydro-2,2,4-trimethylquinoline) = 35 μg/m^3 based a 24-hr averaging time.

Reference:

1. Monsanto Company. 1992. *Follow-up information. Chronic study of Flectol Pastilles antioxidant administered in feed to albino rats (volumes I-III) (final report) with cover letter dated 031292.* Toxic Substance Control Act (TSCA) 8(e) submittal; 89-920000162. (OTS0529777-2).

2. B.F. Goodrich Company. 1978. *Initial submission: Letter from BF Goodrich Company to USEPA submitting an enclosed summary report and a full report on 2,2,4-trimethyl-1,2-dihydroquinoline polymer with attachments.* Toxic Substance Control Act (TSCA) 8(e) submittal; 88-920005368 (OTS0543219).

3. Hodge, HC et al. 1966. *Tests on mice for evaluating carcinogenicity.* Toxicology and Applied Pharmacology; 9:583-596.

4. Panner, BJ and Packer JT. 1961. *Hepatic alterations in rats fed 1,2-dihydro-2,2,4-trimethyl-quinoline, Flectol H.* Proc. Soc. Exptl. Biol. Med; 106:16-19.

5. British Industrial Biological Research Association (BIBRA). 1982. Initial submission: a six-week feeding study with Flectol flakes in rats with cover letter dated 080792. Toxic Substance Control Act (TSCA) 8(e) submittal; 88-920007620 (OTS0545812).

6. Monsanto Company. 1992. Cover letter dated 100892. Preliminary results of Monsanto teratology study No. SB-92-129. Toxic Substance Control Act (TSCA) 8(e) submittal; 89-920000162. (OTS0538311).