MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

June 2, 1998

TO: File for Isophorone (CAS# 78-59-1)

FROM: Michael Depa, Toxics Unit

SUBJECT: Screening Level Determination

The initial threshold screening level (ITSL) for isophorone is 280 µg/m³ (1-hour average). The initial risk screening level (IRSL) for isophorone is 3.7 µg/m³ (annual average). The secondary risk screening level (SRSL) for isophorone is 37 µg/m³ (annual average).

The following references or databases were searched to identify data to determine the screening level: IRIS, RTECS, ACGIH Threshold Limit Values, NIOSH Pocket Guide to Hazardous Chemicals, Environmental Protection Bureau Library, IARC Monographs, CAS Online (1967 - December 30, 1995), National Library of Medicine, Health Effects Assessment Summary Tables, and NTP Status Report. Review of these sources found that EPA has established an RfD for isophorone at 0.2 mg/kg/day (IRIS, 1992). The EPA (IRIS, 1992) also derived a slope factor for isophorone at 9.5×10^{-4} $(mg/kg)^{-1}$ based on data from an NTP oral bioassay (NTP, 1986) where male rats showed an increased incidence of preputial gland carcinomas. The Agency for Toxic Substances and Disease Registry (ATSDR, 1989) developed a chronic oral minimal risk level (MRL) for isophorone of 0.2 mg/kg/day. The MRL was developed to protect against liver and kidney effects observed in rats at the lowest adverse effect level (LOAEL) of 250 mg/kg. The ACGIH and NIOSH have established occupational exposure limits (OELs) for isophorone. The ACGIH ceiling TLV is 28 mg/m³. The NIOSH REL is 23 mg/m³. Summaries of the toxicity of isophorone were published by the ATSDR (1989), EPA (IRIS, 1992) and ACGIH (1991). The molecular weight of isophorone is 138.21g.

Overview of Isophorone Toxicity

The effects of isophorone exposure in humans are irritation of the skin, eyes, nose and throat, and possible dizziness and fatigue (ATSDR, 1989). Occupational exposure limits were established to protect workers against irritation and narcosis (ACGIH, 1998). Isophorone is considered a carcinogen (male rat preputial gland carcinomas) pursuant to Rule 103(c) of the Air Pollution Control Rules promulgated under Article II, Chapter 1, Part 55 of the Natural Resources and Environmental Protections Act, 1994, PA 451.

Animal Studies

The National Toxicology Program (NTP) performed a 2 year gavage study with isophorone in male and female rats and mice (NTP, 1986). NTP stated that under the conditions of these gavage studies, there was some evidence of carcinogenicity of isophorone in male F344/N rats as shown by

the occurrence of renal tubular cell adenomas and adenocarcinomas in animals given 250 or 500 mg/kg/day; carcinomas of the preputial gland were also observed at increased incidence in male rats given 500 mg/kg/day. The incidence of preputial carcinomas in male rats was 0/50, 0/50, and 5/50 for control, low and high dose groups, respectively. There was no evidence of carcinogenicity in female F344/N rats given 250 or 500 mg/kg/day. In male B6C3F1 mice, there was equivocal evidence of carcinogenicity of isophorone as shown by an increased incidence of hepatocellular adenomas or carcinomas (combined) and of mesenchymal tumors in the integumentary system in males given 500 mg/kg/day and by an increase in malignant lymphomas in males given 250 mg/kg/day. In female B6C3F1 mice given 250 or 500 mg/kg/day there was no evidence of carcinogenicity of isophorone.

In an inhalation study, groups of 10 male and 10 female Charles River rats were exposed to 250 mg/m³ isophorone 6 hours/day, 5 days/week for 4 weeks, totaling 20 exposures (Hazelton Labs, 1968). The control groups were exposed to filtered air. On Day 1 of exposure, slight nasal bleeding was noted in animals and on Day 6 through 8, a reddish-brown discoloration of the fur surrounding the nasal regions was seen. Otherwise, all animals appeared normal and active throughout the exposure series. The authors stated that gross necropsy findings revealed no clean-cut compound-related abnormalities. Body weight in the male exposure group was significantly lower than the control. The liver to body weight ratio was lower than the control in the isophorone males. Histopathological examination and comparison of the lungs, liver, spleen, kidney, and adrenal glands were performed; however, the results were not presented in detail. It appears from the discussion of the histopathological results that there were no meaningful differences between control and exposed groups. Hematological determinations were made and, "[A]nimals exposed to isophorone exhibited a substantial increase in the percentage of lymphocytes (3.0% among males, and 5.8% among female) and a decrease in percentage of segmented neutrophils (2.8% among males and 5.0% among females)." A LOAEL of 250 mg/m³ was identified from this study based on decreased body weight (females), hematological changes and nasal bleeding.

In an inhalation teratology study, groups of 22 pregnant F344/N rats and 22 pregnant CD-1 mice were dosed with 0, 25, 50, or 115 ppm isophorone (141, 283, 650 mg/m³) on days 6 - 15 of gestation for 6 hours/day (Exxon, 1983). On Day 18 of gestation the mean body weights, corrected for uterine weight, were significantly lower for the 115 ppm exposed mice. Uterine implantations parameters were not effected by exposure to isophorone. Rats were observed to have dose related increases of alopecia and staining of the cervical/ano-genital areas. No significant differences among control and treated groups were found for any of the fetal external, visceral or skeletal parameters for mice or rats. Rat fetus crown-rump distances were significantly lower for the 115 ppm (650 mg/m³) dose group; however, the authors stated that the result was attributable to two specific female fetuses. The authors stated that if these two fetuses were removed and the remaining data analyzed, there were no differences found. It was decided that it would be inappropriate to analyze the data in this manner. The 115 ppm or 650 mg/m³ dose was determined to be a developmental LOAEL based on decreased crown-rump distances in rat fetuses. A NOAEL of 50 ppm (283 mg/m³) was also identified.

Groups of 10 male Wistar strain albino rats and groups of 10 guinea pigs (strain not mentioned) of mixed sex were exposed to 0, 25, 50, 100, 200, or 500 ppm (0, 141, 283, 265, 1130 or 2826 mg/m³, respectively) isophorone for 30 exposures, 8 hours each (Smyth et al., 1942). Mortality rates in the 25, 50, 100, 200 and 500 ppm dose groups were 0, 0, 12, 17, and 45%, respectively. Chronic conjunctivitis and nasal irritation sometimes proceeding to bloody exudate were caused by repeated exposure to 500 ppm isophorone. The authors stated that animals surviving 100 ppm and upwards

had slower growth. Only animals inhaling 500 ppm isophorone excreted albumin; slight kidney injuries were found microscopically. Animals exposed to isophorone often had pale or brown kidneys, pale livers, congested spleens and lungs, with bile frequently discolored. Animals killed by repeated exposure to isophorone were found to have severely injured kidneys, lungs or both. Kidneys were congested, with dilation of Bowmans's capsule, granular secretion in convoluted tubules, and cloudy swelling, toxic degeneration, or even necrosis of the epithelium of these vessels. The effects observed at the 50 ppm dose level were limited to kidney lesions. The 25 ppm dose level was determined to be a no-observable-effect-level (NOEL). Since kidney lesions are not to be used to quantitate risk (see Discussion below) the NOAEL was determined to be 50 ppm.

Groups of 5 male Wistar rats were given a single dose 0, 1.0, 2.0, 4.0, or 8.0 ml/kg isophorone by gavage (Union Carbide, 1981). Five out of five rats dosed with 8.0 ml/kg died on day 1 of dosing. Four out of five rats dosed with 4.0 ml/kg died on day 1 of dosing and the survivor did not die within the 14 day observation period. One rat given 2.0 ml/kg died, and no rats given 1.0 ml/kg died during the 14 day observation period. The LD50 was determined to be 2.83 (1.85 - 4.34) ml/kg (LD50~2,600 mg/kg).

In a human sensory irritation study, groups of six volunteers were exposed to various concentration of isophorone and asked a series of questions (Hazelton Labs, 1965). A medical examination was performed before the sessions and after each session. The subjects were asked to indicate the following signs: detection of odor, nose irritation, eye irritation, throat irritation, and mask removal. The authors reported that throat irritation occurred at 199 mg/m³ and eye and nasal irritation at 359 mg/m³. At a concentration of 513 mg/m³ one patient removed his face mask after slightly more than two minutes of exposure. A LOAEL of 199 mg/m³ was identified from this study based on throat irritation.

Discussion

After reviewing the literature on the toxicity of isophorone two important issues relating to the quantitation of risk became evident. The first relates to whether or not it is appropriate to use the occurrence of male rat kidney tumors to assess the carcinogenic potential of isophorone. Secondly, it was questioned whether oral toxicity data could be used to quantitate the inhalation risk of isophorone. Each of these issues are discussed below.

Isophorone is one of a number of unique compounds that causes male rat specific kidney tumors. These tumors are associated with the accumulation of α_{2u}-globulin in hyaline droplets in the proximal convoluted tubule in the male rat but not the female rat or most notably male and female mice. The EPA (1991) stated, "Since humans appear to be more like other laboratory animals than like the male rat, in this special situation, the male rat is not a good model for assessing human risk." In 1991, the EPA published guidance concerning the use of kidney lesions for assessing human risk:

1. Male rat renal tubule tumors arising as a result of a process involving α_{2u}-globulin accumulation do not contribute to the qualitative weight-of-evidence that a chemical poses a human carcinogenic hazard. Such tumors are not included in dose-response extrapolations for the estimation of human carcinogenic risk.

2. If a chemical induces α_{2u}-globulin accumulation in male rats, the associated nephropathy is not used as an endpoint for determining non-carcinogenic hazard. Estimates of non-carcinogenic risk are based on other endpoints.

The EPA (1991) provided criteria for evaluating chemically induced male rat renal tubule tumors. Three conditions must be met before it can be concluded that the male rat renal tubule tumors are not to be used for carcinogenic risk analysis. These conditions are listed below.

1. Increased number and size of hyaline droplets in renal proximal tubule cells of treated rats

2. Accumulation of protein in the hyaline droplets is α_{2u}-globulin

3. Additional aspects of the pathological sequence of lesions associated with α_{2u}-globulin nephropathy are present (e.g. linear mineralization and tubule hyperplasia)

Evidence that isophorone meets these conditions set forth by EPA for male rat specific kidney tumors is found in the results from the NTP (1986) study. Non-cancer kidney lesions observed in male rats are presented below.

1. Renal proximal tubular cell hyperplasia was observed in one low dose and four high dose male rats but not in vehicle controls.
2. Epithelial hyperplasia of the renal pelvis was observed in five low dose and five high dose males rats but not in vehicle controls.
3. Mineralization of the renal tubules was observed in male rats (vehicle control 1/50; low dose, 31/50; high dose 20/50).

Additional evidence that isophorone produces male rat specific kidney tumors can be found in a study by Strasser et al. (1988). It was reported that in male rats there was positive evidence for exacerbation of hyaline droplets in renal proximal tubule cells as well as positive evidence for increased renal α_{2u}-globulin levels.

After reviewing the available toxicity database on isophorone it was deemed that isophorone meets the criteria set forth by the EPA (1991) for male rats specific kidney tumors. Therefore, male rat renal tubule tumors and non-neoplastic kidney lesions will not be used to evaluate the toxicity of isophorone.

The toxicity database on isophorone was analyzed to determine if oral toxicity data can be used to quantitate the inhalation risk. Toxicity data obtained from oral studies can be extrapolated to the inhalation pathway when the dose delivered to the target organ does not depend on whether the toxicant enters the body through the gastrointestinal tract or the lungs. In general, when the critical effect is thought to be extrarespiratory or remote and the uptake of the toxicant is not impeded or altered by the respiratory surface then oral toxicity data can be use in an inhalation risk assessment. Isophorone meets both of these criteria. The ATSDR (1989) summarized the absorption of isophorone from inhalation exposure,

> Isophorone was widely distributed to the organs of rats exposed for 4 hours to a concentration of 400 ppm isophorone (Dutertre-Catella 1976), indicating that isophorone is absorbed after inhalation exposure. That isophorone is absorbed by the lungs can also be

> inferred from the systemic toxicity observed in animals following inhalation exposure....Imbriani et al. (1985) measured a blood/air partition coefficient of 2349 for isophorone, indicating that isophorone is absorbed readily from the lungs.

This shows that isophorone is readily absorbed from the lungs and distributed throughout the body. There was no data to indicate that oral toxicity data on isophorone was inappropriate to use to quantitate the inhalation risk.

Development of Screening Levels

Health risk assessments on isophorone were published by the EPA (IRIS, 1992), the ATSDR (1989), and the ACGIH (1992). As mentioned above, the EPA developed an oral reference dose of 0.2 mg/kg/day for isophorone. Similarly, the ATSDR developed a minimal risk level of 0.2 mg/kg. The ACGIH ceiling threshold limit value (C-TLV) for isophorone is 28 mg/m³ and the NIOSH recommended exposure level (REL) for isophorone is 23 mg/m³. The EPA also developed an oral cancer slope factor (q_1*) of 9.5 x 10^{-4} (mg/kg/day)$^{-1}$ based on the increased incidence of preputial gland carcinomas in male rats.

An ITSL can be developed from the oral RfD of 0.2 mg/kg as described in Rule 232(1)(b). According to the rule, the RfD is multiplied by 70kg/20m³ to obtain the ITSL. Doing so, the RfD derived ITSL becomes 700 µg/m³ (24-hour average).

An ITSL can also be developed from an occupational exposure limit or OEL (either the TLV or the REL). Since the ceiling TLV is more protective it was used to develop the ITSL pursuant to Rule 232(1)(c). According to this rule, the ITSL is obtained by dividing the OEL by 100. Dividing the C-TLV of 28 mg/m³ one obtains an ITSL of 0.28 mg/m³ or 280 µg/m³ (based on an 1-hour averaging time).

An IRSL can be developed from the oral slope factor according to Rule 231(3)(f).

$$q_1^* \text{ (µg/m³)}^{-1} = q_1^* \text{ (mg/kg/day)}^{-1} \times (20 \text{ m³}/70 \text{ kg}) \times (1 \text{ mg}/1000 \text{ µg}) \times a/b$$

where "a" is the absorption efficiency by the inhalation route of exposure and "b" is the absorption efficiency by the oral route of exposure. In the absence of data on absorption efficiencies it is assumed that a = b and a/b = 1.

$$q_1^* \text{ (µg/m³)}^{-1} = 9.5 \times 10^{-4} \text{ (mg/kg/day)}^{-1} \times (20 \text{ m³}/70 \text{ kg}) \times (1 \text{ mg}/1000 \text{ µg}) \times 1$$

$$q_1^* \text{ (µg/m³)}^{-1} = 2.7 \times 10^{-7}$$

The IRSL is determined as follows:

$$IRSL = (1 \times 10^{-6})/(q_1^*)$$

$$IRSL = (1 \times 10^{-6})/(2.7 \times 10^{-7} \text{ (µg/m³)}^{-1})$$

$$IRSL = 3.68 \text{ µg/m³}$$

IRSL = 3.7 µg/m³

After evaluating each of the screening levels derived above it was determined that the effects protected by RfD derived ITSL of 700 µg/m³ (24-hour average) would also be protected by the lower ITSL of 280 µg/m³ (1-hour average); therefore, the RfD derived ITSL will not be used to evaluate the emissions of isophorone with regard to Rule 230. Since the SRSL may not be adequate to protect against the irritant effects of isophorone observed in occupational settings, the OEL derived ITSL, the IRSL and the SRSL will be used to evaluate emissions with respect to Rule 230.

The ITSL for isophorone is 280 µg/m³ (1-hour average). The IRSL for isophorone is 3.7 µg/m³ (annual average). The SRSL for isophorone is 37 µg/m³ (annual average).

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