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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: **Sept. 2, 1999**

MEMORANDUM

SUBJECT: ***ISOPHORONE*** - Report of the Hazard Identification Assessment Review Committee.

FROM: P. V. Shah, Ph.D, Toxicologist. P V Shah
Registration Action Branch 1
Health Effects Division (7509C)

THROUGH: Jess Rowland, Co-Chair 9/2/99
and
Pauline Wagner, Co-Chair 9/9/99
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Olga Odiott, Risk Assessor
Registration Action Branch 1
Health Effects Division (7509C)

PC Code: 047401

On June 24, 1999, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) evaluated the toxicology data base of **Isophorone,** established a Reference Dose (RfD) and selected the toxicological endpoints for acute dietary and occupational/residential exposure risk assessments. The HIARC also addressed the potential enhanced sensitivity of infants and children from exposure to Isophorone as required by the Food Quality Protection Act (FQPA) of 1996. The Committee's conclusions are presented in this report.

Members present were:

D. Anderson
W. Burnam
V. Dobozy
K. Hamernik
P. Hurley
M. Ioannou
S. Makris
N. McCarroll
N. Paquette
K. Raffaele
P. Shah
P. Wagner (Co-Chair)
B. Tarplee (Exec. Sec.)

Member(s) in absentia:

T. Levine
J. Rowland (Co-Chair)

Data was presented by P. V. Shah, Toxicologist of Registration Action Branch 1.

Also in attendance were K. Leifer, O. Odiott, K. Whitby, M. Morrow, R. Sandvig, S. Mason and T. Bloem of HED who attended the meeting to discuss exposure, tolerance, and toxicology issues.

Data Presentation:
and
Report Presentation

P. V. Shah
Toxicologist

I. INTRODUCTION

On June 24, 1999, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) assessed the existing Reference Dose (RfD) and established the toxicological endpoints for acute dietary as well as occupational and residential exposure risk assessments pursuant to the Food Quality Protection Act (FQPA) of 1996. The HIARC also addressed the potential enhanced sensitivity of infants and children as required by FQPA.

Isophorone (3,5,5-trimethyl-2-cyclohexen- 1 -one) is a colorless liquid with an odor resembling peppermint. It is a cyclic unsaturated ketone which has irritant properties and possesses a moderate degree of acute toxicity. Isophorone is manufactured commercially by passing acetone over calcium oxide, hydroxide, or carbide at 350^0 C or by heating acetone at 200-250° C under pressure. Isophorone is used as a solvent or cosolvent for polyvinyl and nitrocellulose resins, lacquers, finishes, pesticides, herbicides, and a variety of fats, oils, and gums. Isophorone has recently been patented for use as a woodpecker repellent for utility poles. Isophorone is an excellent solvent for preparing pesticide formulations especially with the anilide and carbamate type of herbicides. Of the total production of Isophorone, 45-65% is used in vinyl coatings and inks, 15-25% in agricultural formulations, 15-30% in miscellaneous uses and exports and 10% as a chemical intermediate.

II. HAZARD IDENTIFICATION

A. Acute Reference Dose (RfD)

Study Selected: 16-Day Range Finding Study in Mice § non-guideline

MRID No.: 00151527

Executive Summary: This range finding study is a part of an NTP carcinogenicity study in mice and rats. No separate DER was prepared for this study, however, the DER on the carcinogenicity study in mice contains detailed information on the range finding study (MRID No. 00151527). In a 16-day study, groups of 5/sex/dose B6C3F1 mice were administered 0, 125, 250, 500, 1000 or 2000 mg/kg/day isophorone in corn oil by gavage, 5 days /week for two weeks(a total of 12 doses). Animals were observed twice daily and weighed on days 0 and 16.

All mice administered 2,000 mg/kg isophorone died before the end of the study (DER Table 2). Final mean body weights relative to those of the controls were 7.8% lower for males that received 1,000 mg/kg and 7.3%-9.3% lower for females that received 250, 500, or 1,000 mg/kg. Male mice lost weight during week 1, probably as a consequence of fighting. Male and female mice that received 1,000 mg/kg staggered after dosing. No compound- related effects were observed at gross necropsy, nor were lesions noted in tissues examined microscopically from the two male and two female mice from the 1,000 mg/kg dose group.

In this range finding study in mice, the **NOAEL of 500 mg/kg/day is based on the clinical signs (staggering) seen at 1,000 mg/kg/day in both male and female mice (LOAEL).**

Dose and Endpoint for Risk Assessment: NOAEL = 500 mg/kg/day based on the clinical signs (staggering) seen in male and female mice at 1,000 mg/kg/day (LOAEL).

Comments about Study/Endpoint: The clinical signs (staggering) which were observed, can be manifested after one or more dose(s). The results of the mechanistic study in rats (MRID No. 44247101) for determination of the mechanism(s) by which Isophorone induces preputial tumors in male rats also supports this NOAEL.

Uncertainty Factor (UF): 100 (includes 10x for inter-species extrapolation, 10x for intra-species variation).

$$\textbf{Acute RfD} = \frac{\textbf{500 mg/kg/day}}{\textbf{100 (UF)}} = \textbf{5.0 mg/kg/day}$$

This Risk Assessment is required.

B. Chronic RfD

Study Selected: 13-Week Feeding Study in Dogs § 82-1b

MRID No.: 00123976

Executive Summary: In a 90-day subchronic oral toxicity study (MRID 00123976), groups of beagle dogs (4 dogs/sex/dose) were given Isophorone (unspecified purity) in gelatin capsules at 0, 35, 75 or 150 mg/kg/day. Hematological, clinical chemistry and urinalysis were measured at the start of the study and at 1, 2 and 3 months.

Administration of Isophorone in the gelatin capsules to male and female dogs at 0, 37, 75 and 150 mg/kg/day for 90 days resulted in no untoward toxicity. Over the 90-day study period, all animals appeared in excellent condition and showed normal responses when handled. There were no significant differences in body weights, food consumption, clinical chemistry parameters, hematological parameters, urinalysis or organ weights between the treatment groups and controls that can be attributed to isophorone treatment at doses up to 150 mg/kg/day. No remarkable gross or microscopic pathologic findings due to the treatment of isophorone were reported.

Based on the data presented in this study, the NOAEL is ≥150 mg/kg/day and LOAEL in this study was not established.

This subchronic oral toxicity study in dogs is classified as **unacceptable (§82-1)** and does not satisfies the Subdivision F guideline requirements. This study can be **upgraded to acceptable** study provided the deficiencies cited at the end of this DER are met. The deficiencies were:

1. The purity of the test article and capsule isophorone concentration were either not determined or reported. This omissions have major impact on overall acceptability of study results.

2. No signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided. However, this study was conducted prior to GLP regulations.

3. The high dose was not adequate because it did not produced any toxic effects. This deficiency may have major impact on overall acceptability of study results.

4. Individual food consumption data and food efficiency was not reported.

5. Platelets count and Clotting time, electrolytes, blood creatinine and some organ necropsy findings was not presented, however, this omission does not affect interpretation of the study.

Dose and Endpoint for Establishing RfD: NOAEL =150 mg/kg/day based on lack of effects at the highest dose tested (HTD).

Uncertainty Factor(s): 1000 (10x for intra-species variability and 10x for inter-species extrapolation. An additional factor of 10 was applied for the use subchronic study for the chronic end point and for the lack of chronic study and uncertainty about 90-day dog study).

$$\textbf{Chronic RfD} = \frac{\textbf{150 mg/kg/day (NOAEL)}}{\textbf{UF= 1000}} = \textbf{0.15 mg/kg/day}$$

Comments about Study/Endpoint/Uncertainty Factor: This study was selected for the chronic RfD in IRIS database. However, the uncertainty factors were modified to cover deficiencies such as the use subchronic study for the chronic end point, for the lack of chronic study and uncertainty about the quality of 90-day dog study, given the deficiencies cited in the DER..

This risk assessment is required.

C. Occupational/Residential Exposure

1. Dermal Absorption

No dermal absorption studies are available for Isophorone. No suitable studies for extrapolation of dermal absorption are available in the database. In addition, many solvents are known to penetrate the skin rapidly by altering the physical property of the skin. Therefore, dermal absorption of Isophorone is assumed to be 100% (of the oral equivalent dose).

Dermal Absorption Factor: 100% (of the oral equivalent dose).

2. Short-Term Dermal - (1-7 days)

Study Selected: 16-Day Range Finding Study in Mice § non-guideline

MRID No.: 00151527

Executive Summary: See Acute RfD

Dose and Endpoint for Risk Assessment: NOAEL = 500 mg/kg/day based on the clinical signs (staggering) seen in male and female mice at 1,000 mg/kg/day (LOAEL).

Comments about Study/Endpoint: The clinical signs (staggering) were observed, can be manifested after one or more dose(s). The results of the mechanistic study (MRID No. 44247101) also supports this NOAEL.

Since an oral NOAEL was used, a dermal absorption factor of 100% should be used for route-to-route extrapolation for this risk assessments.

This risk assessment is required.

3. Intermediate-Term Dermal (7 Days to Several Months)

Study Selected: 13-Week Feeding Study in Dogs § 82-1b

MRID No.: 00123976

Executive Summary: See Chronic RfD

Dose and Endpoint for Risk Assessment: NOAEL =150 mg/kg/day based on lack of toxicity at the highest dose tested (HTD).

Comments about Study/Endpoint: This study is appropriate for this exposure period of concern (7-days to several months).

Since an oral NOAEL was used, a dermal absorption factor of 100% should be used for route-to-route extrapolation for this risk assessments.

This risk assessment is required.

4. Long-Term Dermal (Several Months to Life-Time)

Study Selected: 13-Week Feeding Study in Dogs § 82-1b

MRID No.: 00123976

Executive Summary: See Chronic RfD.

Dose and Endpoint for Risk Assessment: NOAEL =150 mg/kg/day based on lack of toxicity at the highest dose tested (HTD).

Comments about Study/Endpoint: This study was selected for the chronic RfD. However, the uncertainty factors were modified to cover deficiencies such as the use subchronic study for the chronic end point and for the lack of chronic study and uncertainty about the quality of the 90-day dog study.

Since an oral NOAEL was used, a dermal absorption factor of 100% should be used for route-to-route extrapolation for this risk assessments.

This risk assessment is required.

5. Inhalation Exposure. (ANY-TIME PERIOD)

Study Selected: Prenatal Developmental Study- Rats § 83-3

MRID No.: 00151531

Executive Summary: In a developmental toxicity study (MRID 00151531), isophorone [96.8%] was administered by the whole-body inhalation route, as a vapor, to groups of 22 mated female Fischer 344 rats at dose levels of 0, 25, 50, or 115 ppm *[0, 0.14, 0.28, or 0.64 mg/L]* from days 6 through 15 of gestation.

There were no treatment-related effects in mortality or cesarean parameters compared to controls. Clinical signs of toxicity were seen in the mid- and high-dose animals and consisted of alopecia [1 control female, 14 mid-dose, and 21 high-dose females] and brown staining in the ano-genital or cervical region [1 control female, 11 mid-dose, and 22 high-dose]. Food consumption was significantly decreased [$p<0.01$] in high dose animals during days 6 through 20 of gestation [-10%] and days 0 through 20 of gestation [-8%]. Body weight was significantly [$p<0.05$] decreased in high-dose females at gestation days 12 [-6%] and 15 [-7%]. **The maternal LOAEL for rats is 50 ppm (0.28 mg/L), based on an increased incidence of clinical signs. The maternal NOAEL is 25 ppm (0.14 mg/L) [LDT].**

At the high dose, significantly [$p<0.05$] decreased fetal crown-rump distance [-3%] in female fetuses in comparison to controls was observed in this study. However, the HIARC (June 24, 1999) recommended that this effect is marginal and fetal weight was not affected. **Therefore, the rat developmental NOAEL was established at ≥115 ppm (≥0.64 mg/L). The developmental LOAEL was not established.**

The developmental toxicity study is classified as **acceptable/Guideline and** satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3*(a)*).

Dose/Endpoint for Risk Assessment: Maternal NOAEL 25 ppm (0.14 mg/L) based on an increased incidence of clinical signs (alopecia, brown staining in the anogenital or cervical region) seen at 50 ppm (0.28 mg/L).

Comments about Study/Endpoint: Since no other inhalation studies are available, the HIARC recommended that this dose be used for Short, Intermediate and Chronic exposure risk assessments. This study also included exposure to mice but the results were not appropriate for use in this risk assessment because the NOAEL in this species was higher [50 ppm (0.28 mg/L)] than the rats.

This risk assessment is required.

D. Margins of Exposures for Occupational/Residential Exposure Risk Assessments

For occupational exposure risk assessments, a MOE of 100, 300 and 1000 is required for Short, Intermediate and Long Term dermal exposure risk assessments, respectively. A MOE of 100 is adequate for inhalation exposure risk assessments. The MOE for residential (dermal and inhalation) exposure risk assessments will be determined by the FQPA Safety Committee.

For Short-term occupational expsoure risk assessments, a MOE of 100 is required for (10x for intra-species variability and 10x for inter-species extrapolation) because a NOAEL was selected for this risk assessment.

For Intermediate-term occupational expsoure risk assessments, a MOE of 300 is required for (10x for intra-species variability, 10x for inter-species extrapolation and 3x for uncertainty about the quality of the 90 day dog study) because a NOAEL was selected for this risk assessment.

For Long-term occupational expsoure risk assessments, a MOE of 1000 is required for occupational expsoure risk assessments (10x for intra-species variability and 10x for inter-species extrapolation. An additional factor of 10 was applied for the use subchronic study for the chronic end point and for the lack of chronic study and uncertainty about 90-day dog study) because a NOAEL was selected for this risk assessment.

E. Recommendation for Aggregate (Food, Water and Dermal) Exposure Risk Assessments

For acute aggregate exposure risk assessment, combine the high end exposure values from food + water and compare it to the acute RfD.

For **short-, intermediate- and long-term** aggregate exposure risk assessment, the Aggregate Risk Index (ARI) should be used due to differences in the MOEs required for these exposure scenarios:

- For acute and chronic, use uncertainty factor of 100 and 1000, respectively.
- For short-, intermediate- and long-term dermal, use MOEs of 100, 300 and 1000, respectively.
- For short-, intermediate- and long-term inhalation, use MOE of 100.

Calculate separate MOEs for each route because of different toxicological endpoint and MOEs are different for each duration and route.

$$\text{Aggregate MOE}_{(\text{total})} = \frac{1}{1/\text{MOE}_{(\text{oral})} + 1/\text{MOE}_{(\text{dermal oral equivalent})} + 1/\text{MOE}_{(\text{inhalation})}}$$

III. CLASSIFICATION OF CARCINOGENIC POTENTIAL

For cancer end point use the Q* value of 6.08×10^{-4} based on preputial gland adenomas or carcinomas in male rats.

1. Combined Chronic Toxicity/Carcinogenicity Study in Rats

1.1 Chronic Toxicity Study in Rats

No suitable studies are available either in the in-house database or in open literature.

1.2 Carcinogenicity Study in Rats

MRID No. 00151527

Executive Summary In a carcinogenicity study (MRID 00151527), isophorone (94% and 97%) was administered by gavage to 50 F344/N rats/sex/dose in corn oil at dose levels of 0, 250 or 500 mg/kg/day, 5 days/week for 103 weeks.

Doses selected for the 2-year study were based on a 16-day study in which male and female rats received doses of 0-2,000 mg/kg per day and on 13-week study in which male and female rats received doses ranging from 0 to 1,000 mg/kg per day by gavage in corn oil. No chemically related gross or histopathologic effects were observed in the 16-day or 13week study, but 1/5 high dose male rats, 4/5 high dose female rats died during the 16-day study. During the 13-week study, 1/10 high dose female rats died. The high dose for the 2-year study was set at 500 mg/kg per day for each sex of rats based mainly on the deaths in the 13-week study.

Throughout the 2-year study, the mean body weights of the high dose male rats averaged 5% lower than those of the vehicle controls. During the second year, the mean body weights of the female high dose rats averaged 8% lower than those of the vehicle controls. The survival of high dose male rats was significantly lower than that of the vehicle controls after week 96 (final survival: vehicle control, 33/50; low dose, 33/50; high dose, 14/50). The survival of dosed female rats was poor (30/50; 23/50; 20/50), due in part to 20 gavage related accidental deaths of dosed animals.

Dosed male rats showed a variety of proliferative lesions of the kidney (tubular cell hyperplasia: 0/50; 1/50; 4/50; tubular cell adenoma: 0/50; 0/50; 2/50; tubular cell adenocarcinoma: 0/50; 3/50; 1/50; epithelial hyperplasia of the renal pelvis: 0/50; 5/50; 5/50). Dosed male rats also exhibited increased mineralization of the medullary collecting ducts (1/50; 31/50; 20/50), and low dose male rats showed a more severe nephropathy than is commonly seen in aging F344/N rats. Carcinomas of the preputial gland were increased in high dose male rats (0/50; 0/50; 5/50). With the exception of a moderate increase in nephropathy (21/50; 39/50; 32/50), female rats did not show chemically related increased incidences of neoplastic or nonneoplastic lesions.

Under the conditions of this 2-year gavage study, 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 per day. Carcinomas of the preputial gland were also observed at an increased incidence in male rats given 500 mg/kg. There was no evidence of carcinogenicity in female F344/N rats given 250 or 500 mg/kg per day.

The US EPA IRIS database indicates a Q_1^* of 9.5×10^{-4} using the $^2/_3$'s scaling factor. The new Q_1^* of 6.08×10^{-4} reflects the change from use of the $^2/_3$'s to the $^3/_4$'s scaling factor in 1994. The unit risk, Q_1^* $(mg/kg/day)^{-1}$, of isophorone based upon the male rat preputial gland carcinoma tumor rates is 6.08×10^{-4} in human equivalents (converted from animals to humans by use of the $^3/_4$'s scaling factor, Memo L. Brunsman, 1999).

This carcinogenicity study in the F344/N rats is **acceptable/non guideline and does not satisfy** the guideline requirement for a carcinogenicity study (83-2*(a)*) in rats.

The Systemic Toxicity No Observed Adverse Effect Level (NOAEL) was not determined in the NTP study.

The Systemic Toxicity Lowest Observed Adverse Effect Level (LOAEL) = was not determined in the NTP study.

Discussion of Tumor Data: The EPA IRIS database has classified Isophorone as a Group C carcinogen (possible human carcinogen) based on no data in humans and limited evidence of carcinogenicity of one tumor type in one sex of one animal species as shown by an increase of perputial gland carcinoma in male rats. The apparent renal tubular cell tumor in the male rat is associated with alpha-2u-globulin, and is considered to be of questionable relevance to humans.

Preputial gland carcinomas were observed in five high dose male rats. The absence of this neoplasm in vehicle controls or in the low dose group and the low historical incidence (12/1,094, 1%) in corn oil vehicle controls in previous NTP 2-year studies suggest that this effect may be chemically related. Two clitoral gland adenomas were seen in low dose female rats, providing further evidence for the association of isophorone exposure with this type of neoplasm. However, the prepuce and clitoris are among those tissues examined microscopically only when a neoplasm is visible to the prosector. Therefore, although the neoplasms observed in this study were rather large, the actual incidence of all types of proliferative lesions of the prepuce or clitoris is not known, since only seven animals were sampled for histopathologic examination (five high dose male rats and two low dose female rats). The diagnosis or the actual occurrence of preputial tumors has been sporadic in vehicle controls in previous NTP studies. The number of preputial gland adenomas or carcinomas (combined) in corn oil vehicle controls in previous studies has ranged from zero to seven; five were observed in the corn oil vehicle controls in the one previous comparable study performed at this laboratory (Appendix F, Table FI, of the NTP report). These factors make it difficult to relate with certainty the occurrence of preputial gland carcinomas with exposure to isophorone. Nonetheless, this finding should not be discounted.

Adequacy of the Dose Levels Tested . Despite the overall low survival of dosed and vehicle control dosed female rats, the NTP considers the present 2-year study to be an acceptable assessment of the chronic toxicity and carcinogenicity of isophorone. The lower survival of dosed female rats was due in part to a greater incidence of gavage accidents in the dosed animals. Although 14 gavage-related deaths occurred in the high dose female rats, survival remained above 50% through week 98. The survival of high dose male rats was lower than that of the vehicle control and low dose animals after week 96. The reduced survival is most likely a chemically related effect; however, it probably had a minimal impact even on the incidence of late-developing neoplasms because the steep decline in survival occurred late in the study. The high dose of 500 mg/kg isophorone appeared appropriate for male rats. Although the survival of high dose male rats was significantly lower than that of the vehicle controls, the decline in survival occurred late in the study.

2. Carcinogenicity Study in Mice

MRID No. 00151527

Executive Summary In a carcinogenicity study (MRID 00151527), Isophorone (94% and 97%) was administered via gavage to 50 B6C3F1albino mice/sex/dose in corn oil at dose levels of 0, 250 or 500 mg/kg/day, 5 days per week for 103 weeks.

Doses selected for the 2-year study were based on the results of a 16-day study in which mice of each sex received doses of 0-2,000 mg/kg per day and on a 13-week study in which mice of each sex received doses ranging from 0 to 1,000 mg/kg per day by gavage in corn oil. No chemically related gross or histopathologic effects were observed in the 16-day or 13week study, but all high dose male and female mice died during the 16-day observation period. During the 13-week study, 3/10 high dose female mice died. The high dose for the 2-year study was set at 500 mg/kg per day for each sex of mice, based mainly on the deaths in the 13-week study.

During the second year, the mean body weights of the high dose female mice averaged 5% lower than that of the vehicle controls. The survival of male mice was also low (16/50; 16/50; 19/50), but there was a significant trend toward increased survival of dosed female mice relative to that of the vehicle controls (26/50; 35/50; 34/50).

In high dose male mice, isophorone exposure was associated with increased incidences of hepatocellular adenomas and carcinomas (18/48; 18/50; 29/50) and of mesenchymal tumors of the integumentary system (fibroma, fibrosarcoma, neurofibrosarcoma, or sarcoma: 6/48; 8/50; 14/60). An increased incidence of lymphomas or leukemias was noted in low dose male mice (8/48; 18/50; 5/50). Coagulative necrosis (3/48; 10/50; 11/50) and hepatocytomegaly (23/48; 39/50; 37/50) were observed more frequently in the livers of dosed male mice than in vehicle controls. No compound-related neoplastic or nonneoplastic lesions associated with isophorone exposure were seen in female mice.

Under the conditions of this 2-year gavage study, there was equivocal evidence of carcinogenicity of isophorone in male B6C3F1 mice as shown by an increased incidence of

hepatocellular adenomas or carcinomas (combined) and of mesenchymal tumors in the integumentary system in animals given 500 mg/kg per day and by an increase in malignant lymphomas in animals given 250 mg/kg per day. There was no evidence of carcinogenicity of isophorone in female B6C3F1 mice given 250 or 500 mg/kg per day.

This carcinogenicity study in the B6C3F1 mice is **acceptable/non guideline and does not satisfy** the guideline requirement for a carcinogenicity study (83-2*(b)*) in mice.

Discussion of Tumor Data Isophorone exposure was associated with a marginal increase in the incidence of neoplastic lesions of the liver and the integumentary and lymphoreticular systems of male mice. Nonneoplastic lesions were also observed in the liver and adrenal cortex of dosed male mice.

The incidence of hepatocellular adenomas or carcinomas (combined) was greater in the high dose male mice than that in the vehicle controls (vehicle control, 18/48; low dose, 18/50; high dose, 29/50). Although the incidence in vehicle controls was similar to the historical average for adenomas and carcinomas in vehicle controls in previous NTP corn oil studies (32.4%), the incidence in the high dose group was nearly double this and exceeded the greatest incidence reported in vehicle controls in previous NTP studies. Isophorone-exposed male mice also had an increased incidence of heptocytomegaly and coagulative necrosis of the liver. Acute and/or chronic inflammation of the liver was also noted in 11 of the high dose male mice but in only 1 vehicle control. However, there was no evidence of chemically related nonneoplastic or neoplastic liver lesions in female mice, and hepatocytomegaly was observed less frequently in the dosed female animals (vehicle control, 32/50; low dose, 21/50; high dose, 9/50).

The incidence of mesenchymal tumors of the integumentary system was also significantly elevated in high dose male mice compared with that of vehicle controls by trend analyses and pairwise comparison (fibroma, fibrosarcoma, neurofibrosarcoma, or sarcoma: vehicle control, 6/48; low dose, 8/50; high dose, 14/50). The incidence of these neoplasms in high dose male mice exceeded the mean incidence in historical controls by over fivefold and is therefore regarded as a chemically related effect.

Adequacy of the Dose Levels Tested Despite the overall low survival of dosed and vehicle control male mice, the NTP considers the present 2-year study an acceptable assessment of the chronic toxicity and carcinogenicity of isophorone. Fighting apparently contributed to the low survival of the group-housed male mice, and low survival reduces the power of the study to detect changes in the tumor incidences; however, since fighting occurred in all groups, nearly equal numbers of vehicle control and high dose male mice remained at risk for development of neoplastic and nonneoplastic lesions throughout the study. The survival of dosed female mice was notably greater than that of the vehicle controls. Of the 25 vehicle control female mice that died of natural causes before or during the terminal kill, 15 had at least one type of neoplasm, but no single cause could be identified to account for the accelerated mortality of this group after week 87.

3. Classification of Carcinogenic Potential

The EPA IRIS database has classified Isophorone as a Group C carcinogen (possible human carcinogen) based on no data in humans and limited evidence of carcinogenicity of one tumor type in one sex of one animal species as shown by an increase of perputial gland carcinoma in male rats. The apparent renal tubular cell tumor in the male rat is associated with alpha-2u-globulin, and is considered to be of questionable relevance to humans. There was an apparent increase in hepatocellular and integumentary tumors in male mice which was complicated by high mortality. No increases were seen in females of both rats and mice.

This quantitative risk assessment for Isophorone is based on the Agency's risk assessment found in the IRIS database which indicates a Q_1^* of 9.5×10^{-4} using the $^2/_3$'s scaling factor. The new Q_1^* of 6.08×10^{-4} reflects the change from use of the $^2/_3$'s to the $^3/_4$'s scaling factor in 1994[1]. The dose levels used from the 103-week gavage study were 0, 250, and 500 mg/kg/day via corn oil, 5 days/week. The corresponding tumor rates for the male rat preputial gland carcinomas were 0/49, 0/46, and 5/44, respectively (Memorandum from Lori L. Brunsman to P. V. Shah, entitled, "Isophorone Quantitative Risk Assessment (Q_1^*) Based On Fischer 344/N Rat Gavage Study Using mg/kg b.w.$^{\wedge 3}/_4$/day Cross Species Scaling Factor" dated June 10, 1999).

IV. MUTAGENICITY

The NTP has tested this compound in several genetic toxicity assays. Isophorone was tested for mutagenicity in the Salmonella/microsome assay and in the mouse lymphoma L6178Y/TK"- assay. Isophorone was not mutagenic in strains TA100, TA1535, TA1537, or TA98 of *Salmonella typhimurium* in the presence or absence of Aroclor 1254-induced Sprague-Dawley male rat or male Syrian hamster liver S9. Isophorone was weakly mutagenic in the mouse lymphoma L5178Y/TK"- assay in the absence of S9; it was not tested in the presence of S9. Isophorone was also found to induce sister-chromatid exchanges in Chinese hamster ovary cells in the absence of S9, but this effect was eliminated in the presence of Aroclor 1254-induced male rat liver S9. In addition, isophorone did not induce chromosomal aberrations in the presence or absence of S9 in Chinese hamster ovary cells (NTP report No. 291, 1986). It was negative in the *Drosophila* (sex-linked recessive lethal/reciprocal translocation assay and sister chromatid exchanges assay (NTP on line).

V. FQPA CONSIDERATIONS

1. Neurotoxicity:

No acute or subchronic neurotoxicity studies are available.

2. Developmental Toxicity

In a developmental toxicity study (MRID 00151531), isophorone [96.8%] was administered by the

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[1]See memo - Deriving Q_1^*s Using the Unified Interspecies Scaling Factor, P.A. Fenner-Crisp, Director, HED, 7/1/94.

whole-body inhalation route, as a vapor, to groups of 22 mated female CD-1 mice and 22 mated female Fischer 344 rats at dose levels of 0, 25, 50, or 115 ppm *[0, 0.14, 0.28, or 0.64 mg/L]* from days 6 through 15 of gestation.

In mice, there were no treatment-related effects in mortality, clinical signs, food consumption, or cesarean parameters. Gestation day 18 mean body weights, corrected for uterine weight, were significantly lower [$p<0.05$, -6%] at 115 ppm in comparison to controls. **The maternal LOAEL for mice is 115 ppm (0.64 mg/L), based on decreased gestation day 18 body weight, corrected for uterine weight. The maternal NOAEL for mice is 50 ppm (0.28 mg/L).**

In rats, there were no treatment-related effects in mortality or cesarean parameters compared to controls. Clinical signs of toxicity were seen in the mid- and high-dose animals and consisted of alopecia [1 control female, 14 mid-dose, and 21 high-dose females] and brown staining in the ano-genital or cervical region [1 control female, 11 mid-dose, and 22 high-dose]. Food consumption was significantly decreased [$p<0.01$] in high dose animals during days 6 through 20 of gestation [-10%] and days 0 through 20 of gestation [-8%]. Body weight was significantly [$p<0.05$] decreased in high-dose females at gestation days 12 [-6%] and 15 [-7%]. **The maternal LOAEL for rats is 50 ppm (0.28 mg/L), based on an increased incidence of clinical signs. The maternal NOAEL is 25 ppm (0.14 mg/L) [LDT].**

In mice, the developmental LOAEL was not established. The developmental NOAEL is 115 ppm [HDT].

Significantly [$p<0.05$] decreased fetal crown-rump distance [-3%] in female fetuses in comparison to controls was observed in this study. However, the HIARC (June 24, 1999) recommended that this effect is marginal and fetal weight was not affected. **Therefore, the rat developmental NOAEL was established at $\geq$115 ppm ($\geq$0.64 mg/L). The developmental LOAEL was not established.**

The developmental toxicity study in *rats and mice* is classified as **acceptable/Guideline and** satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3*(a)*).

3. Reproductive Toxicity

No reproductive toxicity studies are available.

4. Additional information from the literature

There are no additional neurotoxicity studies or developmental neurotoxicity studies via inhalation or any other routes available from the published literature.

5. Determination of Susceptibility

In prenatal developmental toxicity studies following *in utero* exposure in rats and mice, there was no evidence of developmental effects being produced in fetuses at lower doses as compared to maternal animals.

6. Recommendation for a Developmental Neurotoxicity Study:

The HIARC recommended the requirement of a developmental neurotoxicity study to be kept as **reserved,** pending the results of acute and subchronic neurotoxicity studies.

7. Recommendation of the FQPA Safety Factor:

Based on the hazard assessment alone, the HIARC recommends to the FQPA Safety Factor Committee that the additional 10x factor should be **retained** for dietary risk assessments because:

(I) Lack of availability of non-rodent developmental toxicity studies.

(ii) Lack of availability of a two generation reproduction toxicity study in rats.

(iii) Lack of availability of acute or subchronic toxicity studies.

(iv) Concern based on structure-activity, information on similar class of compounds and human experience suggest neurotoxic effects.

The final recommendation on the FQPA safety factor is made during risk characterization by the FQPA Safety Committee.

VI. HAZARD CHARACTERIZATION

Isophorone (3,5,5-trimethyl-2-cyclohexen- 1 -one) is a colorless liquid with an odor resembling pep-permint. It is a cyclic unsaturated ketone which has irritant properties and possesses a moderate degree of acute toxicity. It is completely miscible with hydrocarbons, alcohols, esters, ethers, ketones. It has a water solubility of 1.2% at 20^0C and partition coefficient value of 1.67 (20^0C). Isophorone is an excellent solvent preparing pesticide formulations especially with the anilide and carbamate type of herbicides. Of the total production of Isophorone, 45-65% is used in vinyl coatings and inks, 15-25% in agricultural formulations, 15-30% in miscellaneous uses and exports and 10% as a chemical intermediate.

Isophorone vapor causes irritation of eye, nose and throat at 25 ppm in humans. It causes smarting of the skin and first degree burns on short exposure and may cause second degree burns on long exposure. Nausea, headache, dizziness, faintness, inebriation and a feeling of suffocation was experienced by a few subjects exposed to 200 and 400 ppm isophorone these levels. Signs of irritation and narcosis were also experienced at 40 and 85 ppm but decreased with time indicating that a tolerance to the warning property of this vapor may develop.

The database of Isophorone is incomplete and many of the guideline toxicological studies are not available. Acute RfD of 5.0 mg/kg/day was derived from the 16-Day Range Finding Study in Mice. This study was considered appropriate by the committee because clinical signs (staggering) observed in the study occurred after a single exposure (dose).

There is a moderate confidence in the chronic RfD derived from the Subchronic feeding study (capsules) in dog, which was used by IRIS. In this critical study, there was no evidence of compound related effects at the highest dose tested (150 mg/kg/day). This study was conducted prior to GLP Regulations and FIFRA testing guidelines. However, this study included almost all components of the current testing guidelines. The chronic RfD was determined by adding a 3x factors for the uncertainty about the study.

Carcinogenicity study in mice and rats appeared to be positive for carcinogenicity. Based on the results of this study, NTP concluded that 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 per day; carcinomas of the preputial gland were also observed at an increased incidence in male rats given 500 mg/kg. There was no evidence of carcinogenicity in female F344/N rats given 250 or 500 mg/kg per day. In mice, there was equivocal evidence of carcinogenicity of isophorone in male B6C3F1 mice as shown by an increased incidence of hepatocellular adenomas or carcinomas (combined) and of mesenchymal tumors in the integumentary system in animals given 500 mg/kg per day and by an increase in malignant lymphomas in animals given 250 mg/kg per day. There was no evidence of carcinogenicity of isophorone in female B6C3F1 mice given 250 or 500 mg/kg per day. The EPA's IRIS database also classified Isophorone as a Group C carcinogen (possible human carcinogen) and limited evidence of carcinogenicity in animals. The IRIS calculated Q* value of $9.5x\ 10^{-4}$ mg/kg/day was based on preputial gland carcinoma in male rats seen in the NTP study.

Isophorone was not mutagenic in strains TA100, TA1535, TA1537, or TA98 of *Salmonella typhimurium* in the presence or absence of Aroclor 1254-induced Sprague-Dawley male rat or male Syrian hamster liver S9. Isophorone was weakly mutagenic in the mouse lymphoma L5178Y/TK"assay in the absence of S9; it was not tested in the presence of S9. Isophorone was also found to induce sister-chromatid exchanges in Chinese hamster ovary cells in the absence of S9, but this effect was eliminated in the presence of Aroclor 1254-induced male rat liver S9. In addition, isophorone did not induce chromosomal aberrations in the presence or absence of S9 in Chinese hamster ovary cells (NTP report No. 291, 1986). It was negative in the *Drosophila* (sex-linked recessive lethal/reciprocal translocation assay and sister chromatid exchanges assay.

VII. DATA GAPS

The HIARC has identified the need for a non-rodent developmental toxicity study, 2-Generation reproduction study and acute and subchronic neurotoxicity studies via route(s) of concern as a data gap. In addition, the HIARC has **reserved** the requirement for a developmental neurotoxicity study, pending the results of acute and subchronic neurotoxicity studies.

VIII. ACUTE TOXICITY

GDLN	Study Type	MRID	Results	Tox Category
81-1	Acute Oral (rat)	00152029	LD_{50} = 2.83 mL/kg (1.85- 4.34)	
81-2	Acute Dermal (rabbit)	00152029	LD_{50} = 1.26 mL/kg (0.772- 2.06)	
81-3	Acute Inhalation (rat)	00152029	LC_{50} > saturated vapors No mortality, clinical signs	
81-4	Primary Eye Irritation (rabbit)	00152029	moderate severe corneal fluorescein staining, slight corneal dullness, moderate iritis	
81-5	Primary Skin Irritation (rabbit)	00152029	slightly irritating to one out of four rabbits (grade 2)	
81-6	Dermal Sensitization			

IX. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary	NOAEL=500 UF = 100	Based on increased in clinical signs (staggering) seen at 1000 mg/kg/day (LOAEL, HDT).	16-Day Range Finding Study in Mice
	Acute RfD = 5.0 mg/kg/day		
Chronic Dietary	NOAEL =150	LOAEL was not established. No evidence toxicity was observed at 150 mg/kg/day [HDT] (NOAEL).	Subchronic Toxicity-Dog
	UF = 1000	**Chronic RfD = 0.15 mg/kg/day**	
Short-Term (Dermal) [a]	Oral NOAEL= 500	Based on increased in clinical signs (staggering) seen at 1000 mg/kg/day (LOAEL, HDT).	16-Day Range Finding Study in Mice
Intermediate-Term (Dermal) [a]	Oral NOAEL=150	LOAEL was not established. No evidence toxicity was observed at 150 mg/kg/day [HDT] (NOAEL).	Subchronic Toxicity-Dog
Long-Term (Dermal) [a]	Oral NOAEL=150	LOAEL was not established. No evidence toxicity was observed at 150 mg/kg/day [HDT] (NOAEL).	Subchronic Toxicity-Dog
ANY TIME POINTS (Inhalation)	NOAEL= 25 ppm (0.14 mg/L)	Based on an increased incidence of clinical signs (alopecia, brown staining in the anogenital or cervical region) seen at 50 ppm.	Developmental Toxicity Study-Rats

a = The use of a 100% dermal absorption factor is required since an oral dose was selected.