EVIDENCE ON THE CARCINOGENICITY OF

ALLYL ISOVALERATE

DRAFT

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Reproductive and Cancer Hazard Assessment Section
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PREFACE

The Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code 25249.5 *et seq*.) requires that the Governor cause to be published a list of those chemicals "known to the state" to cause cancer or reproductive toxicity. The Act specifies that "a chemical is known to the state to cause cancer or reproductive toxicity … if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity." The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency. The "state's qualified experts" regarding findings of carcinogenicity are identified as the members of the Carcinogen Identification Committee of the OEHHA Science Advisory Board (22 CCR 12301).

Allyl isovalerate was assigned a final priority of 'high' carcinogenicity concern and placed on the Final Candidate list of chemicals for Committee review on August 6, 1999. A public request for information relevant to the assessment of the evidence on the carcinogenicity of this chemical was announced on August 6, 1999, in the *California Regulatory Notice Register*. No information was received as a result of this request.

This draft document *Evidence on the Carcinogenicity of Allyl Isovalerate* was developed to provide the Committee with relevant information for use in its deliberations. It reviews the available scientific evidence on the carcinogenic potential of allyl isovalerate. A public meeting of the Committee to discuss this evidence is scheduled for December 18, 2001. At this meeting it is expected that the Committee will render an opinion on whether allyl isovalerate has been clearly shown to cause cancer. Written public comment on the document should be submitted to OEHHA by December 4, 2001, in order to be considered by the Committee in advance of the meeting. During the December 2001 meeting, the public will have an opportunity to present verbal comments to the Committee.

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EXECUTIVE SUMMARY

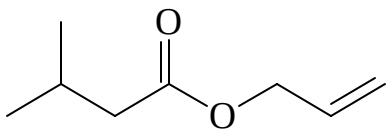
Allyl isovalerate is a branched-chain allyl ester compound with potential human exposures stemming from its use as an approved flavoring agent. Quantitative estimates of current exposure to allyl isovalerate are difficult since the addition of the compound to food products may be up to the level required to produce its intended effect, reporting is not required, and available data are somewhat dated. Based on levels reported in some food, personal hygiene and cosmetics products, exposure to low levels of the compound may be expected to be widespread.

There is evidence for the carcinogenicity of *allyl isovalerate*, with the development of hematopoietic tumors in male rats and female mice treated for two years by oral gavage. Further evidence includes observations of genotoxicity in short-term tests in mammalian cells and possible metabolism to a carcinogenic compound.

2 INTRODUCTION

2.1 Identity of Allyl Isovalerate

Molecular Formula: $C_8H_{14}O_2$



Molecular Weight: 142.2

CAS Registry No.: 2835-39-4

Chemical Class: Allyl ester

Synonym: AIV; allyl 3-methylbutyrate; 2-propenyl 3-methylbutanoate; 2-propenyl isopentanoate; ally isopentanoate; European Inventory of Existing Commercial Chemical Substances (EINECS) No. 220-609-7; Flavouring Extract Manufacturers' Association (FEMA) No. 2045

Boiling point: 152.3°C (Chemfinder, 2001); 89-90°C (HSDB, 2001)

2.2 Occurrence and Use

Allyl isovalerate is a colorless liquid primarily used as a flavoring agent and as "raw material for fragrances in cosmetics, lotions, and perfumes and in certain food products" (IARC, 1999a; Opdyke, 1979). Its organoleptic characteristics have been described as bittersweet with a "fruit-like (apple, cherry) aroma"; the compound is not known to occur naturally (Furia and Bellanca, 1975; Burdock, 1995).

Allyl isovalerate is listed by the U.S. Food and Drug Administration (U.S. FDA, 2000) among "synthetic flavoring substances which may be safely used in food" and may be "used in the minimum quantity required to produce their intended effect, and otherwise in accordance with all the principles of good manufacturing practice." While the compound has been used since the 1950s, the Flavouring Extract Manufacturers' Association (FEMA) granted allyl isovalerate GRAS (generally recognized as safe) status in 1965 (Opdyke, 1979, citing FEMA, 1965). Allyl isovalerate appears on the European Communities register of flavoring substances used in or on foodstuffs and on the inventory of ingredients employed in cosmetic products (European Communities, 1999; European Communities, 2000).

While available information appears somewhat dated, allyl isovalerate levels have been reported in various food products, including alcoholic and non-alcoholic beverages (8.6, 10 and 11 ppm), ice cream and ices (18 and 35 ppm), hard and soft candy (22 and 37 ppm), baked goods (14–48 ppm), gelatins and puddings (1.0 and 12 ppm) (Furia and

Bellanca, 1975, citing Bedoukian, 1967; Burdock, 1995). For personal hygiene products, another report indicated "usual" concentrations in terms of percent allyl isovalerate in the final product as follows: 0.003 % in soap (0.2 % maximum), 0.0003 % in detergent (0.002 % maximum), 0.0015 % in creams and lotions (0.007 % maximum), and 0.05 % in perfume (0.08 % maximum) (Opdyke, 1979). More recent exposure data have not been located and it is not clear whether use patterns changed following the publication of the National Toxicology Program (NTP) studies (NTP, 1983b), which provide the most complete available assessment of the toxicity of allyl isovalerate. Overall, it may be expected that since the chemical is approved in the U.S. for use as an organoleptic agent, widespread exposure at low levels may occur.

Allyl isovalerate may be synthesized by "direct esterification of allyl alcohol with isovaleric acid under azeotropic conditions" (HSDB, 2001, citing Arctander, 1969). A single manufacturer of allyl isovalerate has been identified (Bell Flavors and Fragrances of Northbrook, IL; production site in Los Angeles, CA), and U.S. production has only been reported since 1973, although current production was not evaluated (IARC, 1985; HSDB, 2001). U.S. production estimates for 1977 and 1979 were 1000 lbs (HSDB, 2001). The NTP (1983b) cites a report showing U.S. production in 1980 exceeded 1000 lbs. (U.S. ITC, 1981). IARC reported that there was no commercial production in western Europe or Japan (IARC, 1985).

3 DATA ON ALLYL ISOVALERATE CARCINOGENICITY

Long-term carcinogenicity studies of allyl isovalerate have been conducted in both rats and mice. Allyl isovalerate has also been tested for genotoxicity in *Salmonella* reverse mutation assays in multiple strains as well as other short-term *in vitro* tests in mammalian cells.

3.1 Epidemiological Studies of Carcinogenicity in Humans

No data on long-term effects of human exposure to allyl isovalerate were found in a recent search by OEHHA.

3.2 Carcinogenicity Studies in Animals

3.2.1 Long-term Gavage Studies in Rats

F344/N rats (50/sex/group) were treated by oral gavage with 0, 31 or 62 mg/kg allyl isovalerate in corn oil five days per week for 103 weeks (NTP, 1983b). The test substance was found to consist of 95.6% of the ester and 0.37% of the free acid. Two minor impurities were identified by vapor-phase chromatography (1.7% and 1.5%), although they were not further characterized.

No significant differences in survival were observed between treated and control groups at any dose.

Among male rats, a statistically significant increase in mononuclear cell leukemia (called "monocytic leukemia" in the pathology report) was observed in the high-dose group, with a statistically significant positive trend with increasing dose (see Table 1). Additionally, two malignant lymphomas were observed in male rats in the high-dose group, with none in the control or low-dose group.

The incidence of combined preputial gland adenomas and carcinomas was significantly increased in the low-dose group of male rats by Fisher's exact test. The incidence of preputial gland adenomas was significantly increased in the low-dose group relative to the controls by the Life Table test. There were no significant positive trends for this endpoint.

No tumor incidences among allyl isovalerate-treated female rats were significantly increased relative to control groups. A positive trend by Life Table analysis for combined leukemias (primarily mononuclear cell leukemias) was observed among female rats, although this trend was only marginally positive by exact analysis for linear trend ($p = 0.082$; see Table 1).

Table 1. Tumors in F344/N rats treated with allyl isovalerate for 103 weeks (NTP, 1983b).

Tumor Site and Type		Dose (mg/kg)			Trend test[a]
		0	31	62	
Males					
Hematopoietic	Mononuclear cell leukemia	1/50	4/50	7/50[b,c]	0.021
	Malignant lymphoma, histiocytic	0/50	0/50	2/50	n.s.
Preputial gland	Adenomas	0/50	4/50[c]	1/50	n.s.
	Combined adenomas / carcinomas	0/50	5/50[b,c]	2/50	n.s.
Females					
Hematopoietic	Mononuclear cell leukemia	4/50	6/50	8/49	n.s.
	Leukemia, not otherwise specified	0/50	0/50	1/49	n.s.
	Leukemia, combined	4/50	6/50	9/49	0.082[d]
	Malignant lymphoma, histiocytic	1/50[e]	0/50	1/49[f]	n.s.
	Thymoma	0/41	0/43	1/39	n.s.

[a] Exact analysis for linear trend (a generalization of the Fisher Exact test) (n.s. = not significant, $p > 0.10$).
[b] Significantly increased incidence with respect to controls by Fisher's Exact test ($p < 0.05$).
[c] Significantly increased incidence with respect to controls by Life Table and Incidental Tumor test ($p < 0.05$).
[d] Significant positive trend by Life Table test ($p = 0.05$).
[e] Observed in mesenteric lymph node.
[f] Observed in mesentery.

Historical control tumor incidences among F344 rats from studies of at least 104 weeks with groups of animals of 35 or more from seven testing laboratories were reported by NTP (see Table 2 below).

As a subset of the historical control incidences reported in Table 2 below, the control incidences among the five corn oil gavage studies conducted at the same facility as the allyl isovalerate studies (Southern Research Institute) were also presented in the NTP report. Among male rats the control incidence of all leukemias ranged from 2 to 10 % (overall 4%, including the allyl isovalerate study) and among female rats 4 to 16 % (overall 10 %).

In male rats, the significantly increased incidence of mononuclear cell leukemia (7/50 = 14 %) was within the range of control incidences for all leukemias at the seven NTP study facilities, although it was outside the range for studies conducted at the same facility.

Table 2. Historical control incidences of tumors in F344/N rats in seven study locations prior to 1982 (NTP, 1983b).

Tumor Type	Males		Females	
	Range	Overall*	Range	Overall*
Leukemia	2 – 24 %	9.6 %	2 – 42 %	13.2 %
Lymphoma	0 – 8 %	1.4 %	0 – 6.1 %	1.5 %
Preputial gland adenoma	0 – 14 %	1.6 %	–	–
Preputial gland carcinoma	0 – 14 %	1.7 %	–	–
Preputial gland adenocarcinoma	0 – 8 %	0.5 %	–	–

* Total tumors / total animals; total animals = 999 rats of each sex.

Significant *decreases* in the incidences of pituitary adenomas and C-cell thyroid carcinomas were observed among low-dose male rats. The trend was significant only for the decrease in pituitary adenomas.

3.2.2 Long-term Gavage Studies in Mice

$B6C3F_1$ mice (50/sex/group) were treated by oral gavage with 0, 31 or 62 mg/kg allyl isovalerate in corn oil five days per week for 103 weeks, as described in the rat studies above (same test substance and dosing regimen, although mice received 10 ml/kg corn oil *vs.* 5 ml/kg for rats; NTP, 1983b). The hybrid $B6C3F_1$ mouse strain used in these studies was found to have originated from a C3H parental strain with a high degree of variance at one to three genetic loci (85% incidence). Although the potential difference between these mice and those derived from a more genetically homogeneous source is not known, within these two-year mouse studies, control and treated groups were matched with respect to origin, *i.e.*, they had the same expected degree of genetic heterogeneity.

Overall survival in the low-dose group of female mice was significantly lower than the control group ($p = 0.001$, by the Kaplan and Meier method). NTP suggested this increase in mortality was caused by a "suppurative lesion of the ovary/uterus which often spread to other areas in the abdominal cavity." No other significant differences in survival among groups of mice were observed.

Among male mice, the incidences of squamous cell papillomas of the gastric mucosa showed a positive trend by Incidental Tumor analysis ($p = 0.048$; by exact test for linear trend, $p = 0.056$). The increase in incidence was not statistically significant in either treated group relative to the control animals. Epithelial hyperplasia of the gastric mucosa also increased among treated male mice, with incidences of 1/50, 1/50, and 7/48 in the control, low-, and high-dose groups, respectively. Two squamous cell papillomas of the gastric mucosa were also observed among high-dose female mice and a single papilloma was observed in the control group. The incidences of epithelial hyperplasia of the gastric mucosa in female mice were 0/50, 2/50, and 3/50 in the control, low-, and high-dose groups, respectively.

Among female mice, a significant increase in malignant lymphoma (all types) was observed in the high-dose group relative to the control group (see Table 3). A marginally significant increase in histiocytic malignant lymphomas was also observed in the high-dose group. For both of these endpoints a statistically significant positive trend was observed.

Table 3. Tumors in B6C3F$_1$ mice treated with allyl isovalerate for 103 weeks (NTP, 1983b).

Tumor Site and Type		Dose (mg/kg)			Trend test[a]
		0	31	62	
Males					
Gastric mucosa	Squamous cell papilloma	0/50	1/50	3/48	0.056[b]
Hematopoietic	Malignant lymphoma, all	4/50	6/50	8/50	n.s.[c]
Females					
Gastric mucosa	Squamous cell papilloma	1/50	0/50	2/50	n.s.
Hematopoietic	Malignant lymphoma, lymphocytic	5/50	5/50	4/50	n.s.
	Malignant lymphoma, histiocytic	0/50	1/50	4/50[d]	0.026
	Malignant lymphoma, mixed type	6/50	5/50	10/50	n.s.
	Malignant lymphoma, all	11/50	11/50	18/50[e]	0.071[f]

[a] Exact analysis for linear trend (a generalization of the Fisher Exact test) (n.s. = not significant, $p > 0.10$).
[b] Significant positive trend by Incidental Tumor test ($p = 0.048$); marginally significant positive trend by Life Table test ($p = 0.068$).
[c] Marginally significant positive trend by Incidental Tumor test ($p = 0.077$).
[d] Marginally statistically significant increase in incidence with respect to controls ($p = 0.059$ by Fisher's Exact test; $p = 0.052$ by Life Table test).
[e] Statistically significant increase in incidence with respect to controls by Life Table test ($p = 0.034$) (by Fisher's Exact test, $p = 0.093$).
[f] Significant positive trend by Incidental Tumor test ($p = 0.037$) and Life Table test ($p = 0.026$).

Historical control tumor incidences among B6C3F$_1$ mice from studies of at least 104 weeks with groups of animals of 35 or more from seven testing laboratories were reported by NTP (see Table 4 below).

As a subset of the historical control incidences reported in Table 4, the control incidences of hematopoietic tumors among the five corn oil gavage studies conducted at the same facility as the allyl isovalerate studies (Southern Research Institute) were also presented in the NTP report. Among male mice the incidence of all lymphomas ranged from 6 to 18 % (overall 11 %, including the allyl isovalerate study) and among female mice 10 to 22 % (overall 15 %).

Table 4. Historical control incidences of tumors in B6C3F$_1$ mice in six study locations prior to 1982 (NTP, 1983b).

Tumor Type	Males		Females	
	Range	Overall*	Range	Overall*
Stomach tumors (combined)	0 – 2 %	0.6 %	n.a.	n.a.
Lymphoma	4.2 – 34.7 %	20.1 %	0 – 18.2 %	12.2 %

* Total tumors / total animals; total animals = 881 to 1007 mice of each sex.
n.a. = not available in NTP (1983).

In female mice, the increased incidence of malignant lymphomas in the high-dose group (18/50 = 36%) is outside the range of control incidences reported for all seven testing facilities (0 to 18.2 %) as well as for the same testing facility the allyl isovalerate studies were conducted (10 to 22 %). The incidence of gastric papillomas in the high-dose group of male mice (3/48 = 6.25 %) is outside the range of incidences for historical controls for the six study locations (0 to 2 %).

The genetic variability in the strain of mouse noted in the study description has a potential bearing on the background incidence of tumor types. OEHHA is not aware of any data on spontaneous tumor incidences in B6C3F$_1$ mice with comparable genetic variability from which to evaluate this possibility.

Statistically significant *decreases* in tumor incidence with respect to control animals were observed at several sites and doses, including hepatocellular carcinomas and combined liver tumors in low-dose and high-dose male mice, alveolar/bronchiolar adenomas and combined lung tumors among high-dose male mice, and follicular cell adenomas of the thyroid among low-dose male mice. Among female mice, a significant decrease in the incidence of pituitary adenomas was observed in the low-dose group relative to the control group.

Discussion of Carcinogenicity Studies in Animals

In summary, allyl isovalerate induced mononuclear cell leukemias in male rats exposed for two years, with a significant increase in incidence and a significant positive trend. In female rats, a significant positive trend for combined leukemias was also observed, although no significant increase in incidence over control animals was observed for any given dose group. Female mice showed a significant increase in the incidence of malignant lymphomas, with a significant positive trend.

NTP concluded that "[u]nder the conditions of these studies, allyl isovalerate was carcinogenic for F344/N rats and B6C3F$_1$ mice, causing increased incidences of hemato-poietic system neoplasms (mononuclear cell leukemia in male rats and lymphoma in female mice)."

IARC concluded that allyl isovalerate was "not classifiable as to its carcinogenicity to humans" (Group 3), based upon the absence of epidemiological data and limited evidence of carcinogenicity in experimental animals (IARC, 1999a).

3.3 Other Relevant Data

3.3.1 Genetic Toxicology

Allyl isovalerate was not found to be mutagenic in reverse mutation assays in *Salmonella typhimurium* strains TA 98, 100, 1535, or 1537, either with or without metabolic activation (NTP, 1983b; reported in Mortelmans *et al.*, 1986). Metabolic activation was achieved with Arochlor-1254-induced liver S-9 preparations from both Sprague-Dawley rats and Syrian hamsters.

Cytogenetic toxicity testing was performed on Chinese hamster ovary cells using 95.6% pure allyl isovalerate in DMSO (Gulati *et al.*, 1989). Sister chromatid exchanges were weakly increased in the presence of S9 metabolic activation (from Aroclor 1254-induced male Sprague-Dawley rats) and more strongly in its absence, with a ~1.5-fold increase at the highest dose tested. The authors noted that this effect was observed only at cytostatic doses. Chromosomal aberrations were also observed in the presence of S9 metabolic activation at *in vitro* doses of 300-500 µg/ml allyl isovalerate, with a ~10-fold increase in percent of cells with aberrations.

According to a review by Tennant *et al.* (1987) and as reported by NTP (2001a), allyl isovalerate tested positive in a mouse lymphoma forward mutation assay (L5178Y cells) in the absence of metabolic activation. The lowest concentration reported to produce a positive result was 100 µg/ml allyl isovalerate. The primary reference for these data was not located.

Allyl isovalerate was tested for the induction of sex-linked recessive lethal mutations in adult *Drosophila melanogaster* exposed by feed or injection (Woodruff *et al.*, 1985). No evidence of sex-linked recessive lethal mutations was found.

Tests for morphological transformation of BALB/c mouse 3T3 cells by allyl isovalerate were deemed negative by the study's authors (Matthews *et al.*, 1993).

NTP concluded in 1983 that there was "considerable evidence … of genotoxic effects of purported allyl isovalerate metabolites [allyl alcohol, acrolein, glycidaldehyde, glycidol], but not of the parent ester" (NTP, 1983b; see Section 3.3.3 Pharmacokinetics and Metabolism below). This conclusion, however, pre-dated the findings of Gulati *et al.* (1989).

Overall, testing of allyl isovalerate for genetic toxicity has been limited to a few diverse tests in bacterial, insect, and mammalian cell systems. Positive results were observed in cytogenetic tests in hamster cells and in a mutagenicity test in mouse cells.

3.3.2 Structure-Activity Comparisons

Several compounds with allyl functional groups have been tested in long-term bioassays in rodents (see Figure 1 below). Allyl isothiocyanate administered by gavage to rats for two years produced transitional-cell papillomas in the urinary bladders of males (NTP, 1982). Female rats in the same set of experiments showed some evidence of increasing subcutaneous fibrosarcomas (positive trend). NTP concluded that allyl isothiocyanate was carcinogenic for male rats, with equivocal evidence for female rats. NTP also reports that allyl isothiocyanate generally tested negative in *Salmonella* mutagenicity tests and was positive in tests for chromosomal aberrations and sister chromatid exchanges and in a mouse lymphoma forward mutation assay (NTP, 2001b).

A two-year gavage study of diallyl phthalate in mice showed positive trends for increasing lymphoma and lymphoma or leukemia among males (NTP, 1983a). Positive trends for forestomach papillomas were also observed for both male and female mice. Increased inflammation and hyperplasia of the forestomach were also observed. NTP concluded the evidence of carcinogenicity was equivocal in both sexes of mice. Similarly treated female rats showed some increase in the incidence of mononuclear cell leukemias, although NTP concluded that the evidence was equivocal (NTP, 1985). NTP also reports that diallyl phthalate tested negative in *Salmonella* mutagenicity tests and was positive in tests for chromosomal aberrations and sister chromatid exchanges and in a mouse lymphoma forward mutation assay (NTP, 2001b).

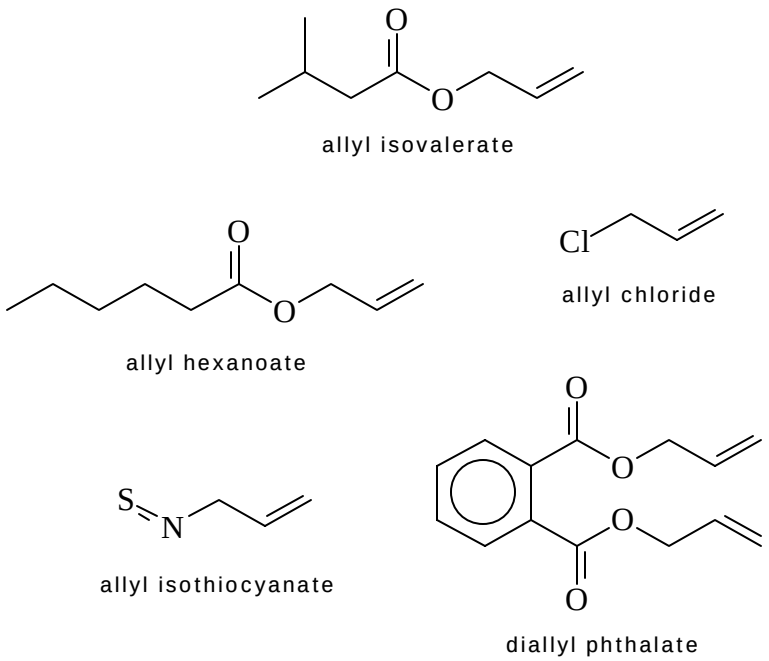
Among mice treated with allyl chloride by gavage for 78 weeks, both males and females showed increased incidences of squamous-cell carcinomas of the forestomach (NCI, 1978). Female mice also showed an increase in the incidence of forestomach papillomas. NCI concluded that there was equivocal evidence of carcinogenicity in male and female mice. Allyl chloride has been found to be mutagenic in a *Salmonella* reverse mutation assay (NTP, 2001b).

Rats fed a diet containing 0.5 % allyl hexanoate for 1½ years developed a low incidence (2/25) of "multiple bile duct adenomas and proliferative changes of the small bile ducts"; another adenoma was identified in an unspecified tissue (Walker, 1991, citing a summary of Bär and Griepentrog, 1967). Information on the inclusion of a control group and tumor incidence in this group was not presented in the brief available summary. Bile duct proliferation ("very slight") was reported in an 18-week study in Osborne-Mendel rats dosed with 65 mg/kg_{bw} allyl hexanoate (Walker, 1991, citing Hagan *et al.*, 1967). No adverse effects, however, were reported in a one-year study of Osborne-Mendel rats (five/sex) fed a diet containing 2500 mg/kg allyl hexanoate (125 mg/kg_{bw}) in which hematological exams were conducted at three, six, and 12 months.

Acrolein, a putative metabolite of allyl isovalerate with an allyl functional group (see below, Section 3.3.3 Pharmacokinetics and Metabolism), showed evidence of carcinogenicity in an oral administration study in male rats, with a significant dose-related increase pancreatic acinar cell tumors (OEHHA, 1997). Additional studies in mice, rats, and hamsters were considered non-positive or inadequate for evaluation. Rats receiving acrolein and uracil intraperitoneally showed an increased incidence of

papillomas of the urinary bladder (IARC, 1995). Acrolein has demonstrated genetic toxicity, inducing mutations in bacteria, sister chromatid exchanges in mammalian cells, and DNA binding *in vitro* (IARC, 1995). OEHHA assigned a "medium" level of carcinogenicity concern to acrolein in prioritizing the compound for review by the Proposition 65 Carcinogen Identification Committee (OEHHA, 1997).

Figure 1. Chemicals with some structural similarity to allyl isovalerate.



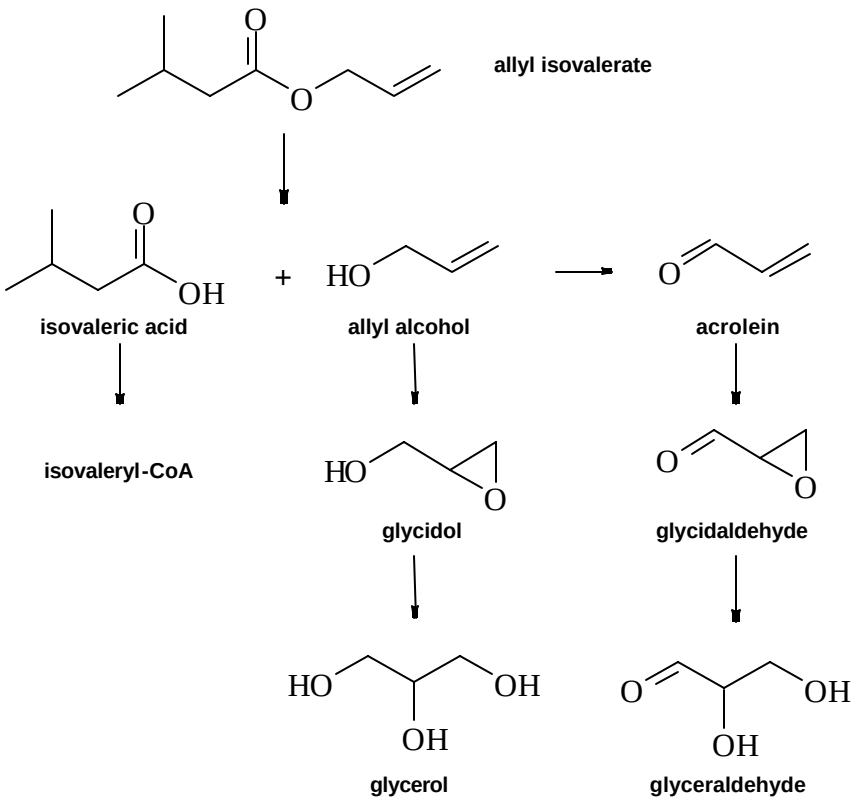
3.3.3 Pharmacokinetics and Metabolism

Information regarding the metabolism of allyl isovalerate is limited. No metabolites of allyl isovalerate have been identified from *in vivo* studies in either experimental animals or humans. According to reviews by the NTP (1983b) and Walker (1991), allyl esters are hydrolyzed *in vivo* to allyl alcohol and an alkyl acid (in the case of allyl isovalerate, isovaleric acid) by esterases of the pancreas, intestinal mucosa, and liver (see Figure 2). The cleavage of allyl esters appears to proceed more rapidly for straight chain allyl esters than for branched chain allyl esters (Walker, 1991, citing Butterworth *et al.*, 1975, and Drake, 1975).

Subsequently, allyl alcohol may be oxidized to acrolein or glycidol (Drake, 1975) and isovaleric acid may be converted to isovaleryl-Coenzyme A (NTP, 1983b, citing Holze and Panten, 1979). The conversion of allyl alcohol to acrolein may be catalyzed by alcohol dehydrogenase. Isovaleryl-CoA is a naturally occurring biochemical also generated from the breakdown of leucine (NTP, 1983b, citing Cohn *et al.*, 1978, Holze and Panten, 1979, and Goodman, 1977). Allyl alcohol and acrolein may each undergo epoxidation to glycidol and glycidaldehyde, respectively. These products may further be oxidized by epoxide hydrolase to glycerol and glyceraldehyde. Alternately, allyl alcohol and acrolein may be oxidized to acrylic acid (not shown in Figure 2). Acrolein also

reacts non-enzymatically with glutathione and other thiol compounds (Walker, 1991, citing Ohno *et al.*, 1985). Glutathione-S-transferases have been shown to conjugate glycidol and glycidaldehyde (Walker, 1991, citing Patel *et al.*, 1980).

Figure 2. Proposed metabolic scheme for allyl isovalerate (adapted from NTP, 1983b).



Glycidaldehyde, a putative metabolite of allyl isovalerate, is on the Proposition 65 list of chemicals known to cause cancer, producing application site tumors in skin painting studies in mice and subcutaneous injection studies in mice and rats (IARC, 1999b). According to IARC: "Repeated inhalation of glycidaldehyde by rats resulted in a reduction in nucleated marrow cells and focal necrosis of liver and kidney. Repeated intravenous injections into rabbits lowered the leukocyte count and the proportion of polymorphonuclear cells." The hematopoietic toxicity of allyl isovalerate has been investigated further and is discussed in more detail below (see Section 3.4 Mechanism).

3.3.4 Pathology

The primary hematopoietic tumors observed in the rat studies were described as monocytic leukemias in NTP's pathology report (NTP, 1983b). Monocytic leukemia is synonymous with the tumor commonly termed mononuclear cell leukemia, also known as large granular lymphocyte (LGL) leukemia, or Tγ lymphocyte leukemia (Stefanski *et al.*, 1990; Ward *et al.*, 1990). These tumors are considered to arise in the spleen, although

this remains uncertain. A human correlate to the mononuclear cell leukemia observed in the Fischer rat has been reported (Reynolds and Foon, 1984; Reynolds and Ward, 1986).

Since the studies on allyl isovalerate, NTP has recommended that in Fischer rats, mononuclear cell leukemia incidence data may be appropriately combined with incidence data for other types of leukemia for risk assessment purposes (McConnell *et al.*, 1986); however, mononuclear cell leukemia incidence data in this rat strain should not be combined with that of malignant lymphomas (all types). Thus, the combined leukemia/lymphoma data presented in the original NTP (1983b) study are not presented in this document.

Three types of malignant lymphoma (lymphocytic, histiocytic, and mixed-type) were observed in treated and control $B6C3F_1$ mice. NTP guidelines for combining rodent neoplasms recommend that malignant lymphomas of all types (but not histiocytic *sarcoma*) in $B6C3F_1$ mice may be combined for risk assessment purposes (McConnell *et al.*, 1986).

3.4 Mechanism

There is no direct evidence indicating the mechanism by which allyl isovalerate induces hematopoietic tumors in experimental animals. Evidence of genotoxicity in short-term tests conducted in mammalian cells, and in particular cytogenetic assays, suggests the potential for DNA damage as a possible mode of action. Metabolic activation *in vivo* to compounds known to cause cancer is another potential mechanism.

According to NTP (1983b), allyl compounds are potential alkylating agents and direct-acting mutagens, with their reactive potential dependent upon the functional group on the saturated carbon. Allyl alcohol is expected to be a weak alkylating agent based upon the alcohol functional group.

Short-term exposure studies in mice have shown indications of hematopoietic toxicity caused by allyl isovalerate (Hong *et al.*, 1988). Female mice (seven/dose) were exposed to 0, 31, 62, or 125 mg/kg for five days per week for two weeks. Increased spleen weights were observed at the high-dose. Total bone marrow cellularity was not affected by treatment; however, the high-dose group showed significantly decreased numbers of granulocyte-macrophage progenitors (CFU-GM) per femur and all dose groups showed reductions in the hematopoietic pluripotent stem cell fraction (CFU-S) . The activity of 6-phosphogluconate dehydrogenase was significantly decreased in the high-dose group, while glucose-6-phosphate dehydrogenase activity, the second enzyme in the hexose monophosphate shunt (an energy source for leukocytes), was decreased, but not significantly. Embden-Meyerhoff and tricarboxylic acid cycle enzymes were not affected by allyl isovalerate treatment. Peripheral red and white blood cell counts were not affected by treatment, nor were lymphocyte function or susceptibility to infection (*L. monocytogenes*, *P. yoelli*, *Listeria*).

Evidence of hematopoietic toxicity in humans from isovaleric acid has been inferred based on observations of a genetic disease called isovaleric acidemia, or "sweaty-feet

syndrome," a rare autosomal recessive disease caused by a deficiency in isovaleryl-coenzyme A dehydrogenase, a component of the metabolism of the amino acid leucine (Cohn *et al.*, 1978; Gilbert-Barness and Barness, 1999). A characteristic of this disease, which generally manifests itself in neonates, is the accumulation of isovaleric acid with clinical symptoms including pancytopenia, ketoacidosis, and coma. Based upon clinical observations during the course of recovery with glycine treatment (glycine conjugates with isovaleric acid and is excreted rapidly), it has been speculated that the hematologic effects were due to "arrested cellular maturation rather than to increased peripheral consumption or decreased stem-cell production" (Cohn *et al.*, 1978). It has been noted that pancytopenia is a common feature in diseases of impaired branched-chain amino acid metabolism (isovaleric, propionic, and methylmalonic acidemia) and may result from the increases in systemic levels of isovalerate, propionate, and methylmalonate (Cohn *et al.*, 1978; Hutchinson *et al.*, 1985). A recent case report described a 19-day old infant who died from the condition (Gilbert-Barness and Barness, 1999). Analysis of the bone marrow at 15 days of age showed "arrest of myelopoiesis at the promyelocyte stage, a decrease in platelets, and pancytopenia, which suggested the diagnosis of promyelocytic leukemia," which was confirmed at autopsy. These authors surmised that "myeloid dysplasia appears to be a direct toxic effect due to isovaleric acid that has resulted in arrest of maturation." Earlier case reports of two infants with the condition have also shown evidence of granulopoietic progenitor cell suppression (Hutchinson *et al.*, 1985).

The possible relationship of hematopoietic toxicity as indicated in the relatively short-term studies in mice exposed to allyl isovalerate and the observations in humans to a carcinogenic mode of action are not established. Toxicity to the hematopoietic system does, however, suggest the possible vulnerability or exposure of this compartment to allyl isovalerate or another toxic intermediate.

4 SUMMARY AND CONCLUSIONS

4.1 Summary of Evidence

In male rats treated by oral gavage for two years with allyl isovalerate, mononuclear cell leukemia was significantly increased. In female mice treated by gavage for two years with allyl isovalerate, malignant lymphomas were significantly increased. Genotoxicity data from mammalian cell assays of allyl isovalerate indicate that the compound has the potential to cause DNA damage as evidenced by the induction of chromosomal aberrations and sister chromatid exchanges in hamster cells, and mutations in mouse cells. Some compounds with allyl functional groups are known to be DNA reactive. Allyl isovalerate is expected to be metabolized to glycidaldehyde, a chemical known to cause cancer.

4.2 Conclusion

There is evidence for the carcinogenicity of allyl isovalerate, with the development of hematopoietic tumors in male rats and female mice treated for two years by oral gavage.

Further evidence includes observations of genotoxicity in short-term tests in mammalian cells and possible metabolism to a carcinogenic compound.

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