

**FOREWORD****INTRODUCTION****Primary Amyl Acetate (Mixed Isomers)**

**Reaction process-derived mixture of two isomers:**

**CAS N°: 628-63-7 (1-pentyl acetate)**

**CAS N°: 624-41-9 (2-methyl-1-butyl acetate)**

## SIDS Initial Assessment Report For

### 22<sup>nd</sup> SIAM

Paris, France, 18-21 April 2006

1. **Chemical Name:** Primary Amyl Acetate (Mixed Isomers)  
Remark: Primary Amyl Acetate (mixed isomers) is a reaction process-derived mixture composed of:  
65% 1-pentyl acetate *and*  
35% 2-methyl-1-butyl acetate
2. **CAS Number:** Reaction process-derived mixture of two isomers:  
628-63-7 (1-pentyl acetate)  
624-41-9 (2-methyl-1-butyl acetate)
3. **Sponsor Country:** United States of America  
National SIDS Contact Point in Sponsor Country:  
Mr. Oscar Hernandez, Director  
U.S. Environmental Protection Agency  
Risk Assessment Division (7403 M)  
1200 Pennsylvania Avenue, NW  
Washington DC 20460  
Phone: (202) 564-7461
4. **Shared Partnership with**
5. **Roles/Responsibilities of the Partners:**
  - Name of industry sponsor /consortium  
American Chemistry Council  
Barbara Francis, Oxo Process Panel  
1300 Wilson Blvd  
Arlington, VA 22209  
Phone: (703) 741-5609
- Process used**  
Robust Summaries/dossiers, the SIAR, and the SIAP were drafted by the Oxo Process Panel's toxicologists. Documents were reviewed by the Oxo Process Panel and the United States Environmental Protection Agency.
6. **Sponsorship History**
  - How was the chemical or category brought into the OECD HPV Chemicals Programme?  
The American Chemistry Council's Oxo Process Panel submitted a test plan and robust summaries for this chemical to the U.S. Environmental Protection Agency in December 2001, under the International Council of Chemical Associations (ICCA) Global Initiative on High Production Volume (HPV) Chemicals Program.

7. **Review Process Prior to the SIAM:** Members of the Oxo Process Panel conducted a comprehensive literature search. Documents were prepared by the Panel and reviewed by industry toxicologists prior to submission to the United States Environmental Protection Agency (U.S. EPA). The EPA conducted reviews of submitted data and offered comments to industry. The EPA submitted documents to OECD for consideration at SIAM 18.
8. **Quality check process:** The quality of existing data was determined using guidance provided in the Manual for Investigation of HPV Chemicals, Chapter 3: Data Evaluation (OECD, 2002).
9. **Date of Submission:**
10. **Date of last Update:** January 2006
11. **Comments:** Primary Amyl Acetate is produced as a commercial reaction process-derived mixture of two isomers and consists of 65% 1-pentyl acetate (CAS 628-63-7) and 35% 2-methyl-1-butyl acetate (CAS 624-41-9). 2-Methyl-1-butyl acetate is not produced except as the minor component of Primary Amyl Acetate.
- Data are presented for Primary Amyl Acetate (mixed isomers) as the mixture of two isomers as well as individually for 1-pentyl acetate, the major component of Primary Amyl Acetate.
- Based on the structure-activity approach, data for 1-propyl acetate (109-60-4), 1-butyl acetate (CAS 123-86-4), and 2-methyl-1-propyl acetate (isobutyl acetate, CAS 78-83-1), are used as structural analogs for the components of Primary Amyl Acetate (mixed isomers) for the endpoint that addresses acute toxicity to fish.

**SIDS INITIAL ASSESSMENT PROFILE**

<b>CAS No.</b>	628-63-7 (pentyl acetate), 624-41-9 (2-methyl-1-butyl acetate)
<b>Chemical Name</b>	Primary Amyl Acetate-Mixed Isomers (commercial reaction process-derived mixture of approximately 65% 1-pentyl acetate (CAS No 628-63-7) and 35% 2-methyl-1-butyl acetate (CAS No 624-41-9))
<b>Structural Formula</b>	CH <sub>3</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -COO-CH <sub>3</sub> (1-pentyl acetate) CH <sub>3</sub> -CH <sub>2</sub> -CH(CH <sub>3</sub> )-COO-CH <sub>3</sub> (2-methyl-1-butyl acetate)

**SUMMARY CONCLUSIONS OF THE SIAR**

Data are presented for Primary Amyl Acetate, which is the reaction process derived mixture of two isomers (1-pentyl acetate, CAS No 628-63-7 and 2-methyl-1-butyl acetate, CAS No 624-41-9) as well as individually for 1-pentyl acetate, the major component of Primary Amyl Acetate. Based on structural similarity with 1-pentyl acetate, toxicity data for 1-propyl acetate (CAS109-60-4) and 1-butyl acetate (123-86-4) were used to support the assessment of acute fish toxicity of Primary Amyl Acetate.

**Human Health**

Primary Amyl Acetate has an acute oral LD<sub>50</sub> value of 12,306 mg/kg bw for female rats and >14,064 mg/kg bw for male rats. The dermal LD<sub>50</sub> in male rabbits was 8359 mg/kg bw, and >14,080 mg/kg bw in females. Rats exposed for 6 hours to substantially saturated Primary Amyl Acetate vapor exhibited difficulty breathing; mortality was 20% among males exposed to 3693 ppm (19,646 mg/m<sup>3</sup>) and 0% among females exposed to 3628 ppm (19,3000 mg/m<sup>3</sup>). Primary Amyl Acetate causes moderate skin and eye irritation. Signs of respiratory irritation were noted in rats exposed to 3620 ppm (19,284 mg/m<sup>3</sup>) Primary Amyl Acetate vapor. Human sensitization test data for Primary Amyl Acetate indicate that it does not induce dermal sensitization; negative results were also obtained in a human photoallergy test.

Information on repeated inhalation exposure is available in rats. Male and female rats were exposed by inhalation to 0, 100, 300, or 500 ppm (0, 532, 1596, 2660 mg/m<sup>3</sup>) for 14 weeks displayed no clinical signs of toxicity and no mortality. All exposed males exhibited a very slight decrease in body weight gain relative to control males. The NOAEC for males and females was 500 ppm (2660 mg/m<sup>3</sup>). In a 13-week inhalation neurotoxicity study, male and female rats were exposed to 0, 300, 600, or 1200 ppm (0, 1596, 3192, 6384 mg/m<sup>3</sup>) Primary Amyl Acetate vapor. No mortality and no signs of toxicity were observed in any group. There were no neurobehavioral effects observed using motor activity measurements, functional observation battery testing, or neuropathological examinations. The NOAEC for neurotoxicity was 1200 ppm (6384 mg/m<sup>3</sup>). There were no abnormalities observed in male and female rats fed Primary Amyl Acetate in their diet at concentrations of 0, 0.1, 0.5, or 1.0% for 90 days; doses in males were equivalent to 0, 68, 320, or 650 mg/kg bw/day; female doses were 0, 74, 350, and 720 mg/kg bw/day. The NOAEC for this study was 1% Primary Amyl Acetate in the diet, or based on the quantity of diet consumed, 650 and 720 mg/kg bw/day in males and females, respectively.

Primary Amyl Acetate has been tested *in vitro* in bacterial as well as animal cell cultures and is not genotoxic in these test systems both in the presence and absence of metabolic activation. Primary Amyl Acetate was negative when tested in a GLP chromosomal aberration assay in rat lymphocytes.

There were no significant effects observed on relative reproductive organ weights, and reproductive organs and tissues were normal in male and female rats exposed for 14 weeks to Primary Amyl Acetate vapor at concentrations up to 500 ppm (2660 mg/m<sup>3</sup>). There were no effects observed on male and female reproductive organs and tissues in rats fed up to 1% Primary Amyl Acetate in the diet for 90 days.

In two developmental toxicity studies, pregnant female rats and rabbits were exposed to Primary Amyl Acetate vapor at concentrations of 0, 500, 1000, and 1500 ppm (0, 2660, 5320, 7980 mg/m<sup>3</sup>) for 6 hours per day during organogenesis. Maternal toxicity was observed in rabbits at 1500 ppm (7980 mg/m<sup>3</sup>) and in rats at all dose levels

as reduced food consumption and decreased maternal body weight gain. The decrease in corrected body weight gain during gestation in rats was significant at 1000 and 1500 ppm (5320 and 7980 mg/m<sup>3</sup>). The NOAEC for maternal toxicity in rabbits and rats was 1000 and 500 ppm (5320 and 2660 mg/m<sup>3</sup>), respectively. Among rabbits exposed between gestation day 6 and 18, no fetal malformations were observed and there was no evidence of developmental toxicity at any exposure level. Among rats exposed between gestation day 6 and 15, no fetal malformations were observed and overall incidence of variations was not increased. Female fetal body weights were reduced at 1000 and 1500 ppm. These fetal body weight decreases were accompanied by increases in one or three skeletal variations, at 1000 and 1500 ppm, respectively as well as two additional variations (one external and one visceral) at 1500 ppm. The NOAEC for developmental toxicity in rabbits and rats was 1500 and 500 ppm (5320 and 2660 mg/m<sup>3</sup>), respectively.

### Environment

The available physicochemical data are adequate to describe the properties of Primary Amyl Acetate. Primary Amyl Acetate has a vapour pressure of 5.73 hPa at 25°C, and a water solubility of 1700 mg/L at 25 °C. It has a boiling point of 146 °C, a measured Log K<sub>ow</sub> of 2.42, and an estimated melting point of -94°C. The vapor pressure of its major component, 1-pentyl acetate, is 4.67 hPa at 25°C and its aqueous solubility is 2,000 mg/L at 20°C. The vapor pressure and aqueous solubility of the second component, 2-methyl butyl acetate were calculated by EPIWIN to be 8.46 hPa and 1,070 mg/L, respectively. The preferred log K<sub>ow</sub> values of 1-pentyl acetate and 2-methyl butyl acetate are 2.34 and 2.26, respectively.

The photochemical removal of 1-pentyl acetate, as mediated by hydroxyl radicals, occurs with a calculated half-life of 34 to 43 hours. Photochemical removal of 2-methyl butyl acetate was calculated to be 41 hours. Primary amyl acetate is biodegradable under aerobic conditions. Primary Amyl Acetate is anticipated to volatilise easily from moving rivers, but only moderately from quiescent lakes and other surface water bodies; the calculated volatilisation half-life for Primary Amyl Acetate is between 3.4 hours from a river and 5.5 days (132 hours) from a lake. Primary Amyl Acetate is not likely to bioaccumulate in food webs. Based on Level III distribution modelling for 1-pentyl acetate, it is estimated that the majority of Primary Amyl Acetate released to the environment will partition into water (26.7%) and soil (66.3%), with a smaller amount in air (6.8%). The stability of primary amyl acetate in water is pH dependent. The predicted half-lives of Primary Amyl Acetate at 25 °C at pH 4, 7, and 9 are 84.8, 138, and 21.9 days, respectively.

Primary Amyl Acetate exhibits low to moderate toxicity to fish, aquatic invertebrates and algae. Primary Amyl Acetate exhibited a 96-hr LC50 in fish of 69 mg/L. In *Daphnia magna*, the 48-hr EC50 was 40.9 mg/L. Finally, in green algae (*Pseudokirchneriella subcapitata*), Primary Amyl Acetate exhibited a 72-hr EC50 of >466 mg/L (growth rate) and a 72-hr EC50 (biomass) of 156 mg/L. Terrestrial data are not available for Primary Amyl Acetate or its components.

### Exposure

Global production of Primary Amyl Acetate was estimated to be less than 10,000 tonnes in 2002. Consumption in 2002 was estimated to be 4,000 tonnes in the US, and 5,300 tonnes in Western Europe. In the United States, Primary Amyl Acetate is manufactured by one company in a continuous process in a closed system using engineering controls, which prevent the escape of liquid or vapors and minimizes release to the environment. Engineering controls are utilized during production, transfer, and loading operations to minimize exposure. The sole manufacturer of Primary Amyl Acetate in the U.S. does not isolate or market 1-pentyl acetate or 2-methyl-1-butyl acetate.

The predominant use of Primary Amyl Acetate is as a direct solvent component in the manufacture of OEM (original equipment manufacturers) factory-applied automotive paints and clearcoats. It is also used as a starting material and process solvent/extractant in the manufacture of pharmaceuticals. Primary Amyl Acetate may be present in cosmetics as a fragrance enhancer at ppm concentrations. The components of Primary Amyl Acetate have been identified in fruit and as naturally-occurring volatiles in cooked food.

Although its individual components may occur naturally in low concentrations in foods, Primary Amyl Acetate does not appear intentionally in food products, and is not approved as a direct or indirect food additive. It is a flammable liquid with a flammable range of 1.1 to 7.5 volume % in air (11,000 – 75,000 ppm) and a flash point of 37°C (99°F). The occupational exposure limit (ACGIH 8-hr TWA) for the components of Primary Amyl Acetate is 50 ppm.

The general population may be exposed to Primary Amyl Acetate as a fugitive emission. The individual

components of Primary Amyl Acetate may also be released from food products, landfills, and sewage.

### **RECOMMENDATION AND RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED**

The following recommendations are applicable only to Primary Amyl Acetate-Mixed Isomers (reaction process-derived product) and not to its individual components.

#### **Human Health:**

The product is currently a low priority for further work. The product possesses properties indicating a hazard for human health (skin, eye and respiratory tract irritation, and potential developmental toxicity). Based on data provided by the sponsor country (relating to production by one producer in the United States which account for an unknown fraction of the global production and relating to the use pattern primarily in the United States), risk management measures are being applied (engineering controls, occupational standards, Material Safety Data Sheets) in occupational settings. Countries may desire to check their own risk management measures for this product to find out whether there is need for additional measures.

#### **Environment:**

The chemical has properties indicating a hazard for the environment (acute aquatic EC/LC50 values between 1 and 100 mg/l). However the product is of low priority for further work for the environment because of its rapid biodegradation and its limited potential for bioaccumulation.

## SIDS Initial Assessment Report

### 1.0 IDENTITY

This document presents data for Primary Amyl Acetate, a reaction process-derived commercial mixture of two isomers, 1-pentyl acetate and 2-methyl-1-butyl acetate. Additional data for the individual components are presented in support of data for the mixture. The individual components are not sponsored in this submission.

#### 1.1 Identification of the Substance

CAS Number:	Mixture of two isomers: 628-63-7 (1-pentyl acetate) 624-41-9 (2-methyl-1-butyl acetate)
IUPAC Name:	Primary Amyl Acetate (mixed isomers)
Molecular Formula:	C <sub>5</sub> H <sub>12</sub> O
Structural Formula:	CH <sub>3</sub> -COO-CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>3</sub> (1-pentyl acetate) CH <sub>3</sub> -COO-CH <sub>2</sub> -CH(CH <sub>3</sub> )-CH <sub>2</sub> -CH <sub>3</sub> (2-methyl butyl acetate)
Molecular Weight:	130.19 g/mol
Synonyms:	amyl acetate, mixed isomers

#### 1.2 Purity/Impurities/Additives

Purity:	>99% weight/weight
Impurities:	1-pentyl alcohol (0.26%), 2-methyl butyl alcohol (0.20%), 2-methyl butyl formate (0.12%), isoamyl acetate (<0.10%)
Additives:	None
Reference:	Cosmetic, Toiletry and Fragrance Association (CTFA), 1986.

#### 1.3 Physico-Chemical Properties

Table 1: Summary of physico-chemical properties for Primary Amyl Acetate (mixture of two isomers) and its components

Test Substance	Primary Amyl Acetate <sup>1</sup>	1-Pentyl Acetate <sup>2</sup>	2-Methyl-1-Butyl Acetate <sup>3</sup>
CAS Registry Number		628-63-7	624-41-9
Physical state	transparent liquid	transparent liquid	transparent liquid
Molecular weight	130.19 g/mol	130.19 g/mol	130.19 g/mol
Melting point	-94 °C (estimated)	-70.8 to -78.5 °C	-56° C (calculated)
Boiling point	146.28 °C	148 to 149.2 °C	140 °C
Relative density	0.87703 g/cm <sup>3</sup>	0.8756 - 0.879 g/cm <sup>3</sup>	
Vapor pressure	5.73 hPa at 25°C	4.665 hPa at 25 °C	8.459 hPa at 25° C (calculated)
Water solubility	1700 mg/L at 25 °C	1,800 - 2,000 mg/L at 20 °C 1730 mg/L at 25 °C	1,070 - 2,360 mg/L at 25° C (calculated)
Partition coefficient n-octanol/water (log value)	2.42 (measured)	2.30 - 3.13 (measured) 2.34 – 2.39 (calculated)	2.26 (calculated)

Test Substance	Primary Amyl Acetate <sup>1</sup>	1-Pentyl Acetate <sup>2</sup>	2-Methyl-1-Butyl Acetate <sup>3</sup>
Henry's law constant	$2.932 \times 10^{-4}$ atm-m <sup>3</sup> /mol	$3.00$ to $3.91 \times 10^{-4}$ atm-m <sup>3</sup> /mol	$0.535$ to $1.016 \times 10^{-3}$ atm-m <sup>3</sup> /mol (calculated)
Flashpoint	37.22 °C (99 °F) closed cup	21 - 25 °C closed cup (70 - 77 °F)	
Flammable range	1.1 to 7.5 volume % (MSDS) <sup>4</sup>	1.1 to 7.5 volume %	
Evaporation rate (butyl acetate = 1.0)		0.3	
Vapor density		4.5 (air = 1)	
Conversion factor	1 ppm = 5.32 mg/m <sup>3</sup>	1 ppm = 5.32 mg/m <sup>3</sup>	1 ppm = 5.32 mg/m <sup>3</sup>

1: Primary Amyl Acetate is a mixture of 1-pentyl acetate and 2-methyl butyl acetate

2: 1-Pentyl acetate is the major component of Primary Amyl Acetate and is present at a concentration of 65%

3: 2-Methyl butyl acetate is the minor component of Primary Amyl Acetate and is present at a concentration of 35%

4: Values for the mixture are taken from the manufacturer's MSDS and are consistent with values for individual component(s)

Primary Amyl Acetate is a liquid at standard temperature and pressure, with a boiling point of 146.3°C and a melting point estimated to be approximately -94° C. It is less dense than water with a specific gravity of 0.87703 g/cm<sup>3</sup> at 20°C (Olson et al., 2006). The solubility limit in water is 1700 mg/L at 25°C (Olson et al., 2006). The vapor density of Primary Amyl Acetate is approximately 4.5 times greater than air; it has a vapor pressure of 5.73 hPa at 25°C (Olson et al., 2006). Using its water solubility, molecular weight, and vapor pressure, a Henry's law constant at 25°C for Primary Amyl Acetate was calculated to be approximately  $2.923 \times 10^{-4}$  atm-m<sup>3</sup>/mole. Primary Amyl Acetate is a flammable liquid with a flash point of 37.22°C and a flammable range of 1.1 to 7.5% by volume.

1-Pentyl acetate is the major component of Primary Amyl Acetate. Given its solubility limits approximately of 2.0 g/L at 20°C, its molecular weight of 130.19 g/mole, and its vapor pressure of 4.66 hPa at 25 °C, a Henry's law constant at 25°C for 1-pentyl acetate was calculated to be approximately  $3.00 \times 10^{-4}$  atm-m<sup>3</sup>/mole. EPIWIN (EPA, 2003) was used to calculate physical properties for 2-methyl butyl acetate using both the molecular structure and the octanol-water partition coefficient. The vapor pressure of 2-methyl butyl acetate is 8.50 hPa at 25°C. Given its solubility limit of 1070 mg/L and its molecular weight of 130.19 g/mole, a Henry's law constant at 25°C was calculated to be approximately  $1.016 \times 10^{-3}$  atm-m<sup>3</sup>/mol. EPIWIN also calculated a Henry's law constant of  $5.35 \times 10^{-4}$  based on its structure.

## 2.0 GENERAL INFORMATION ON EXPOSURE

### 2.1 Production Volumes and Use Patterns

#### 2.1.1 Manufacture and Consumption

Primary Amyl Acetate (mixture of two isomers) is manufactured in an enclosed, continuous process by the esterification of primary amyl alcohol with acetic acid using cobalt or rhodium catalyst. Consumption (amount used) of Primary Amyl Acetate in North America was estimated to be 4.0 thousand metric tons in 2001; in Western Europe, consumption was estimated at 5.3 thousand metric tons (Bizzari et al., 2002). The rest of the world did not consume sufficient Primary Amyl



Acetate to be included in the CEH report. However, total global consumption is anticipated to increase.

### 2.1.2 Uses and Functions

The predominant use (>75% of production in sponsor country) for Primary Amyl Acetate is as a component of factory-applied automotive paints and clearcoats for original equipment manufacturers (OEMs). The remainder of production (24%) is used as a starting materials or process/extractant solvent in the manufacture of pharmaceuticals. When used in pharmaceutical manufacture, Primary Amyl Acetate does not remain in the final product. Less than 1% of total production has the potential for other uses. With the exception of its use as fragrance enhancer, Primary Amyl Acetate is not present in consumer products in the sponsor country. When utilized as a fragrance enhancer, it is typically used at low ppm concentrations. Primary Amyl Acetate is not used as an ingredient in nail polishes and nail polish removers.

A search of a consumer product database found no consumer products in the United States that contain Primary Amyl Acetate as an ingredient (NIH, 2004). The consumer products searched included those in the following categories: automotive, household, pesticides, landscape/yard, personal care, home maintenance, hobby/crafts, and pet care (NIH, 2004). Uses of Primary Amyl Acetate are listed in Table 2.

In the past, Primary Amyl Acetate has been used as a solvent in the manufacture of lacquers, paints and adhesives. In the United States, Primary Amyl Acetate has been replaced by cheaper solvents and is no longer used for these purposes or in consumer and cosmetic products (Personal communication with Dow Chemical).

The components of Primary Amyl Acetate are 1-pentyl acetate and 2-methyl-1-butyl acetate. Some information on uses is available for “amyl acetate.” However, when the isomer(s) are not specified, “amyl acetate” often pertains to 1-pentyl acetate (also known as n-amyl acetate) rather than Primary Amyl Acetate.

Table 2: Uses\* of Primary Amyl Acetate in the United States (USA) and Western Europe

Use	USA	Europe
Direct solvent ( OEM automotive paints)	75	75
Pharmaceutical manufacture (process solvent and extractant)	24	24
Other uses	1	1

\*% of total consumption, data from Bizzari et al., 2002.

As stated above, the sole manufacturer of Primary Amyl Acetate in the U.S. does not isolate or market either of the constituents, 1-pentyl acetate or 2-methyl-1-butyl acetate. Although it is not sponsored as an HPV chemical, 1-pentyl acetate (also known as amyl acetate) has been reported to have several uses, which may lead to additional exposure to this component. 1-Pentyl acetate functions as a solvent for nitrocellulose in nail polishes, enamels, and lacquers, and serves as a solvent in nail enamel removers. It is reportedly used in the dry cleaning industry as a spotting and pre-spotting agent, and in the textile industry as a solvent for printing and finishing fabrics (HSDB, 2002; Clayton and Clayton, 1994, Lewis, Sr. 1993, Elder, 1988). According to the literature, 1-pentyl acetate has been used as a component in pet repellants and as a warning agent in glues. It is approved as a solvent, co-solvent, and/or attractant in pesticide formulations (40 CFR 180.1001). These uses pertain to 1-pentyl acetate, and not to Primary Amyl Acetate.

In research, 1-pentyl acetate is also used as a component of buffer mixtures for antibiotic-DNA binding, and as a weevil attractant (Elder, 1988). Other information indicates 1-pentyl acetate may be used in deodorants, air fresheners, auto body polish/cleaners, and lubricating oils (U.S. EPA, 2005). There are consumer products registered in the SPIN database (Nordic countries) which contain 1-pentyl acetate. In the United States, 1-pentyl acetate is approved by the Food and Drug Administration (FDA) as an indirect food additive for use only as a component of adhesives in food packaging (21 CFR 175.105). 1-Pentyl acetate, has a low odor threshold (Laing, 1982) and a sweet, fruity odor.

1-Pentyl acetate and 2-methyl-1-butyl acetate have been identified in nectarines, apples, and kiwi fruit. The components of Primary Amyl Acetate have also been identified as naturally-occurring volatiles in baked potatoes and fried chicken (HSDB, 2002), banana oil and pear oil (Bisesi, 1994), and liquors (Sax and Lewis, 1987). 1-Pentyl acetate is also found naturally in fruits such as bananas, pears, and apples; it is used in the manufacture of artificial flavoring agents (Sax and Lewis, 1987). Although individual components may occur naturally in very low concentrations in foods, Primary Amyl Acetate does not appear intentionally in food products, and is not approved as a direct or indirect food additive.

## **2.2 Environmental Exposure and Fate**

### **2.2.1 Sources of Environment Exposure**

Primary Amyl Acetate is manufactured by reaction of acetic acid with Primary Amyl Alcohol at elevated temperatures within enclosed equipment, which minimizes release to the environment. Because the reaction mixture is air sensitive, flammable, and has an extremely unpleasant odor, Primary Amyl Acetate is manufactured and stored within enclosed equipment, which prevents the escape of liquid or vapors and minimizes release to the environment. Process wastes are collected for recycling, treatment in biodegradation facilities, or incineration on site. Primary Amyl Acetate is shipped in bulk in tank railcars or trucks. Smaller quantities are transported in drums. Primary Amyl Acetate may be released to the environment during transfer, transportation, storage and disposal activities. Any spillage during loading or handling would be recovered according to facility regulations, and prevented from entering sewers or waterways. Emissions of Primary Amyl Acetate during manufacture are not anticipated to be large, since manufacturing and process equipment are enclosed.

Primary Amyl Acetate is used in industrial applications as a component of OEM automotive paints and as a process solvent or extractant in the manufacture of pharmaceuticals. Primary Amyl Acetate is not expected to be used in touch-up automotive paints due to its high flashpoint and the need for long shelf life.

A search of a consumer product database found no consumer products in the United States that contain Primary Amyl Acetate as an ingredient (NIH, 2004).

### **2.2.2 Photodegradation**

The photodegradation potential for Primary Amyl Acetate, mixed isomers, has not been determined. The photochemical removal of 1-pentyl acetate, the major component of Primary Amyl Acetate, from the troposphere occurs by reaction with hydroxyl radicals. Using pulsed laser photolysis, the 1<sup>st</sup> order rate constant for the reaction of 1-pentyl acetate with hydroxy radicals was determined to be  $7.34 (\pm 0.91) \times 10^{-12}$  cm<sup>3</sup>/molecule-sec at room temperature (El Boudali et al., 1996). The atmospheric photo-oxidation potential for 1-pentyl acetate was estimated using the submodel

AOPWIN (Meylan and Howard, 1993), which calculates a second order half-life with units of  $\text{cm}^3/\text{molecules}\cdot\text{cm}$ . The calculated 2<sup>nd</sup> order rate constant for 1-pentyl acetate was  $7.55 \times 10^{-12} \text{ cm}^3/\text{molecule}\cdot\text{sec}$  at 25 °C. Using a global average tropospheric hydroxyl radical concentration of  $1.5 \times 10^6 \text{ OH molecules/cm}^3$ , and assuming 12 hours of sunlight per day, the estimated half-life of 1-pentyl alcohol in the atmosphere is 34 hours or 1.42 days (Kwok and Atkinson, 1995). When evaluated on structural features, the calculated 2<sup>nd</sup> order rate constant for 1-pentyl acetate was  $6.0225 \times 10^{-12} \text{ cm}^3/\text{molecule}\cdot\text{sec}$  at 25°C, and the estimated tropospheric half-life was 1.766 days or 42.62 hours (AOPWIN, v1.90).

Using AOPWIN (v. 1.91), a second order rate constant was calculated for 2-methyl butyl acetate to be  $6.2987 \times 10^{-12} \text{ cm}^3/\text{molecule}\cdot\text{sec}$  at 25° C. Using the same assumptions as above, the estimated tropospheric half-life for 2-methyl butyl acetate was 1.70 days or 40.7 hours.

Based on photodegradation estimates for its major and minor component, it is anticipated that the half-life of Primary Amyl Acetate in the troposphere is approximately 34 to 43 hours. Other processes, such as photolysis, wet deposition (rain-out), and dry deposition (aerosol formation) are not expected to play an important role in the atmospheric removal of Primary Amyl Acetate.

### 2.2.3 Environmental Stability

#### Stability in Water

The stability of Primary Amyl Acetate in water is pH dependent. The hydrolysis of Primary Amyl Acetate was evaluated according to the OECD Guideline 111 (Davis and Marty, 2004a). The predicted half-life of Primary Amyl Acetate at 25 °C at pH 4, 7, and 9 was 84.8, 138, and 21.9 days, respectively. The predominant isomer in Primary Amyl Acetate is 1-pentyl acetate. The rate constants for the abiotic hydrolysis of 1-pentyl acetate in aqueous solution for solutions with a pH >8 ranged between  $1.025 \times 10^{-1} \text{ L/M}\cdot\text{sec}$  and  $5.93 \times 10^{-2} \text{ L/M}\cdot\text{sec}$  at 25 °C. Based on these rate constants, the half life of 1-pentyl acetate is estimated to be 13.5 days at pH 9, 78.23 days at pH 8, and 2.142 years at pH 7 (HYDROWIN, Version 1.67; ; Howard, 1990). 1-Pentyl acetate is not expected to adsorb significantly to suspended solids and sediment in water (Lyman et al., 1982; Howard, 1990).

#### Stability in Soil

Upon release, the components of Primary Amyl Acetate are expected to volatilize from dry soil surfaces, based on a vapor pressure of 5.3 hPa (The Dow Chemical Company, 2001). An estimated Koc value of 73 was determined for 1-pentyl acetate from a measured log Kow of 2.34 (Howard, 1990); when estimated by chemical structure, the Koc was determined to be 38.5 (PCKOCWIN, v. 1.66). Based on these data, the components of Primary Amyl Acetate are anticipated to have moderate mobility in soil. Primary Amyl Acetate is expected to biodegrade in the soil environment, based upon results from biodegradation studies, which demonstrated degradation rates of 50% or greater within 10 days (See Table 3, Section 2.2.5).

### 2.2.4 Transport Between Environmental Compartments

The vapor pressure of Primary Amyl Acetate is 5.73 hPa at 25°C, with a water solubility value of 1700 mg/L at 25 °C (Olson et al., 2006). According to a model of gas/particle partitioning of semi-volatile organic materials in the atmosphere, Primary Amyl Acetate is expected to exist solely as a vapor under ambient conditions (Daubert and Danner, 1989). A Henry's Law constant was calculated to be  $2.932 \times 10^{-5} \text{ atm}\cdot\text{m}^3/\text{mol}$ , using a molecular mass of 130.19 g/mol and the preferred

vapor pressure and water solubility. For chemicals with a Henry's Law constant  $> 1 \times 10^{-5}$  and  $< 1.0 \times 10^{-3}$  atm-m<sup>3</sup>/mole, volatilization from water is expected to be moderate (Lyman et al., 1982). Primary Amyl Acetate, therefore, would be expected to volatilize from water at a moderate rate. The potential for Primary Amyl Acetate to volatilize from a model river and lake was calculated by EPIWIN (v.3.12), using a water solubility of 1700 mg/L, a vapor pressure of 5.733 hPa, and a Henry's law constant of  $2.932 \times 10^{-5}$  atm-m<sup>3</sup>/mol and default model assumptions. Volatilization half-lives from a model river and lake were 3.4 hours and 5.5 days, respectively.

Upon release of Primary Amyl Acetate into the environment (whether to water, air or soil), several biotic and abiotic fate processes act to disperse and degrade its components. In addition to mixing and dispersing within a water column, Primary Amyl Acetate is subject to biodegradation, volatilization, and photo-degradation. The components released onto soil will be subject to biodegradation but will also exhibit moderate mobility. Airborne vapor is also subject to photo-oxidation as described above. A Henry's law constant for 1-pentyl acetate was estimated to be  $3.00 \times 10^{-4}$  atm-m<sup>3</sup>/mol, this value was calculated from a water solubility of 1700 mg/L (calculated) at 20 °C, a vapor pressure of 3.5 mm Hg (measured), and a molecular weight of 130.19 (HENRYWIN, v. 3.10). A Henry's law constant for 2-methyl butyl acetate was estimated to be  $5.35 \times 10^{-4}$  atm-m<sup>3</sup>/mol when calculated using molecular structure (HENRYWIN, v. 3.10). Volatilization half-lives between 127 and 129 hours have been estimated for 1-pentyl acetate from a model lake, while half-lives of 3 and 6 hours have been estimated for a model river, using preferred values for water solubility and vapor pressure (EPI Suite, v. 3.11; Lyman et al., 1990). Similar half-life values, 122 hours from a model lake and 2.413 hours from a model river, were estimated for 2-methyl butyl acetate using preferred values (EPI Suite, v. 3.11). These results suggest that Primary Amyl Acetate will volatilize fairly easily from fast moving rivers, but only moderately from quiescent lakes and other surface waters.

The measured log K<sub>ow</sub> for Primary Amyl Acetate is 2.42; the preferred log Kow value for 1-pentyl acetate is 2.34 (Meylan and Howard, 1997). The octanol/water partition coefficient suggests that Primary Amyl Acetate would not be expected to partition readily into soil, sediment, or biota. Similarly, 1-pentyl acetate in these media would tend to move to water or ground-water if available. Using EPI Suite (v. 3.11) and PCKOCWIN (v. 1.66), the soil or sediment Koc for 1-pentyl acetate was calculated to be 38.5 L/kg, based on the structural features of the molecule; the Koc for 2-methyl butyl acetate was calculated to be 33.8 L/kg. Based on these soil/sediment partition values, it is anticipated that the components of Primary Amyl Acetate would move fairly readily through soil to groundwater, with little sorption to soil.

Fugacity modeling (Level III) was conducted using EPIWIN (v.3.12) for Primary Amyl Acetate. Input parameters included molecular weight 130.19 g/mol, melting point -94°C, boiling point 146 °C, water solubility 1700 mg/L, log Kow 2.42, and Henry's law constant  $2.932 \times 10^{-5}$  atm-m<sup>3</sup>/mol. Equal releases to air, water and soil were assumed. Media-specific half-lives were selected or calculated by the model. The model used a half-life of 34 hours for atmospheric photo-oxidation, while biodegradation half-lives in water, soil and sediment were 208 h, 416 h, and 1870 h, respectively. Biodegradation half-lives were selected by the model based on the biodegradation submodels within EPIWIN (v.3.12). All other parameters used were the model default values. The results support the above conclusions regarding the movement of Primary Amyl Acetate in the environment with 6.8% distributing to air, 26.7% to water, 66.3% to soil and 0.14% to sediment.

Fugacity modeling (Level III) was conducted for the components of Primary Amyl Acetate using EPI Suite (v.3.11). Input parameters for 1-pentyl acetate included molecular weight 130.19 g/mol, melting point -70.8°C, boiling point 149.2°C, water solubility 1700 mg/L, log Kow 2.34, and Henry's law constant of  $3.55 \times 10^{-4}$  atm-m<sup>3</sup>/mol. Equal releases to air, water and soil were assumed.

Media-specific half-lives were selected or calculated by the model. The model selected  $7.55 \times 10^{-12}$  cm<sup>3</sup>/molecule-sec as the second order rate constant for atmospheric photo-oxidation (Atkinson, 1989), giving a half-life in air of 34.0 hours. Biodegradation half-lives in water, soil and sediment (208 h, 208 h, and 832 h, respectively) were selected by the model based on the biodegradation submodels within EPI Suite (v. 3.11). All other parameters used were the model default values. The results support the above conclusions regarding the movement of 1-pentyl acetate in the environment with 9.38% distributing to air, 37.7% to water, 52.7% to soil and 0.129% to sediment. Modeling was also conducted for 2-methyl butyl acetate. Input parameters included molecular weight 130.19 g/mol, melting point -56°C, boiling point 140°C, water solubility 1070 mg/L, log Kow 2.26, and Henry's law constant of  $5.35 \times 10^{-4}$  atm-m<sup>3</sup>/mol. The model determined a half-life in air of 40.8 hours. Biodegradation half-lives in water, soil and sediment (360 h, 360 h, and 1440 h, respectively) were selected by the model based on the biodegradation submodels within EPI Suite (v. 3.11) with 10.4% distributing to air, 36.9% to water, 52.6% to soil and 0.145% to sediment.

### 2.2.5 Biodegradation

There is one GLP study that assesses the biodegradation of Primary Amyl Acetate according to OECD Guideline 301D. The 28-day aerobic test was conducted using domestic secondary wastewater effluent. Biodegradation of Primary Amyl Acetate measured after 3, 5, 7, 10, 14, 17, 21, and 28 days was 31, 37, 45, 49, 51, 52, 53, and 57% of the ThOD (theoretical oxygen demand), respectively (Davis and Marty, 2004b). These results indicate that Primary Amyl Acetate is indeed biodegradable, and achieved 50% biodegradation after 10 days. In this study, it did not, however, meet the OECD criteria for the designation of “readily biodegradable” (60% biodegradation within a 10-day window following onset of biodegradation).

Primary Amyl Acetate was also evaluated for biodegradability according to American Public Health Association (APHA) Standard methodology, which is similar to OECD Guideline 301D. The 20-day biological oxygen demand (BOD) test was conducted using combined non-adapted domestic sewage treatment plant and industrial treatment seeds. Biodegradation of Primary Amyl Acetate measured after 5, 10, 15, and 20 days was 64, 76, 67, and 72% of the ThOD, respectively. The same investigators assessed the biodegradation of Primary Amyl Acetate by non-adapted raw sewage and synthetic seawater; biodegradation measured after 5, 10, 15, and 20 days was 35, 65, 69, and 87% of the ThOD, respectively. Measured COD was 2.22 mg/mg; the ThOD was reported as 2.33 mg/mg (Price, Waggy, and Conway, 1974).

There are two studies available which evaluated the biodegradation of 1-pentyl acetate, the major component of Primary Amyl Acetate. Pentyl acetate was tested according to APHA Standard methodology, using aerated Lake Superior water as inoculum; biodegradation measured after 10 days was 50% of the ThOD (Vaishnav and Babeu, 1986). The same investigators assessed the biodegradation of 1-pentyl acetate in river water and ground water. Pentyl acetate biodegradation was measured at 5, 10, 15, and 20 days of incubation. The half-life (50% of the ThOD) of pentyl acetate in river water was calculated to be 13 days. The half life in ground water was calculated to be approximately 50 days (Vaishnav and Babeu, 1987).

Table 3: Biotic and Abiotic Degradation of Primary Amyl Acetate and 1-Pentyl Acetate

Substance	Type	Results <sup>1</sup>	Method	Reference
Primary Amyl Acetate <sup>2</sup>	Aerobic	BOD <sub>3</sub> days = 31% ThOD BOD <sub>5</sub> days = 37% ThOD BOD <sub>10</sub> days = 49% ThOD BOD <sub>17</sub> days = 52% ThOD BOD <sub>21</sub> days = 53% ThOD BOD <sub>28</sub> days = 57% ThOD	OECD 301D freshwater, aerobic, domestic wastewater secondary effluent	Davis and Marty, 2004
Primary Amyl Acetate	Aerobic	BOD <sub>5</sub> days = 64% ThOD BOD <sub>10</sub> days = 76% ThOD BOD <sub>15</sub> days = 67% ThOD BOD <sub>20</sub> days = 72% ThOD	APHA Standard Methods, 1971. freshwater, aerobic, domestic/industrial seed	Price, Waggy, and Conway, 1974
Primary Amyl Acetate	Aerobic	BOD <sub>5</sub> days = 35% ThOD BOD <sub>10</sub> days = 65% ThOD BOD <sub>15</sub> days = 69% ThOD BOD <sub>20</sub> days = 87% ThOD	APHA Standard Methods, 1971. seawater microorganisms, non-adapted	Price, Waggy, and Conway, 1974
1-Pentyl acetate <sup>3</sup>	Aerobic	BOD <sub>10</sub> days = 50% ThOD	APHA Standard Methods, 1980 Lake Superior harbor water	Vaishnav and Babeu, 1986, 1987
1-Pentyl acetate	Aerobic	River water: BOD <sub>13</sub> days = 50% ThOD Ground water: BOD <sub>50</sub> days = 50% ThOD	APHA Standard Methods, 1980 River water, ground water	Vaishnav and Babeu, 1987
1-Pentyl acetate	Abiotic, Atmospheric photooxidation	$6.02 \times 10^{-12} \text{ cm}^3/\text{molecule-sec}$ at 25 °C $11.1 \times 10^{-12}$	With a typical tropospheric OH concentration of $1.5 \times 10^6 \text{ molecule/cm}^3$ , the estimated tropospheric half-life for 1-pentyl acetate is 2.83 days	AOPWIN, Version 1.90

1: BOD = Biological Oxygen Demand; ThOD = Theoretical Oxygen Demand

2: Primary Amyl Acetate is a mixture of 65% 1-pentyl acetate and 35% 2-methyl butyl acetate

3: 1-Pentyl acetate is the major component of Primary Amyl Acetate and is present in the mixture at a concentration of 65%

Following onset of biodegradation, all the studies for Primary Amyl Acetate and 1-pentyl acetate, which used surface water seed demonstrated consistent results: biodegradation was approximately 50% or greater within 10 days. In one study, Primary Amyl Acetate did not meet OECD 301D criterion for the “readily biodegradable” classification, which requires reaching 60% biodegradation within a 10-day window. Nevertheless, the data do demonstrate that primary amyl acetate is subject to biodegradation, and based on the weight of evidence (10-day average biodegradation rate was 60% for surface water studies), Primary Amyl Acetate and its major component, 1-pentyl acetate can be considered readily biodegradable in aqueous environments.

## 2.2.6 Bioaccumulation

Utilizing an n-octanol/water partitioning coefficient (Log  $K_{ow}$ ) value of 2.42, a bioconcentration factor (BCF) of 14.57 was calculated for Primary Amyl Acetate using BCFWIN v. 2.15). Using a Log  $K_{ow}$  value of 2.34, a BCF of 11.8 has been calculated for 1-pentyl acetate, the major component of Primary Amyl Acetate (BCFWIN, v. 2.15). Lyman et al (1990) estimated a BCF of 30 for 1-pentyl acetate, using a Log  $K_{ow}$  of 2.3 and a water solubility of 2000 mg/L at 25 °C; a similar BCF value was reported by Howard (1990). A BCF of 11.05 was calculated for 2-methyl butyl acetate using a log  $K_{ow}$  of 2.26 (BCFWIN, v. 2.15). Based on these values, it is anticipated that the components of Primary Amyl Acetate would display a low bioaccumulation potential and would not adsorb to suspended particles and sediment in water (Meylan and Howard, 1997).

## 2.3 Human Exposure

### 2.3.1 Occupational Exposure

Primary Amyl Acetate is used industrially in the manufacture of automotive paints for OEMs (original equipment manufacturers), as a starting material and process solvent/extractant in the manufacture of pharmaceuticals. Primary Amyl Acetate is not expected to be used in automotive touch-up paints due to its high flashpoint.

Primary amyl acetate is manufactured in a continuous process in enclosed equipment. Because the reaction mixture is air sensitive, flammable, and has an extremely unpleasant odor, Primary Amyl Acetate is manufactured and stored within enclosed equipment which prevent the escape of liquid or vapors and minimizes worker exposure and release to the environment.

There is one manufacturer of Primary Amyl Acetate in the United States. In the United States, worker exposure is expected to be lower than the occupational standards, since manufacture and industrial use activities are typically conducted in closed systems using engineering controls. Primary Amyl Acetate is a flammable material and is moderately irritating to the skin and eyes; the neat liquid will discolor upon contact with air. These properties require sufficient engineering and ventilation controls to limit worker exposure.

The exposure limit for the components of Primary Amyl Acetate is 50 ppm or 266 mg/m<sup>3</sup> (ACGIH, 2002). The OSHA Permissible Exposure Limits (PEL) for the 1-pentyl acetate, the major component of Primary Amyl Acetate, is 100 ppm (OSHA, 29 CFR 1910).

Table 4: Exposure limits<sup>1</sup> for Primary Amyl Acetate and 1-Pentyl Acetate<sup>2</sup>

Substance	Type	Exposure Limit	Reference
Primary Amyl Acetate	8-hour TWA	50 ppm (266 mg/m <sup>3</sup> )	ACGIH, 2002
Primary Amyl Acetate	15-min STEL	100 ppm (532 mg/m <sup>3</sup> )	ACGIH, 2002
1-Pentyl Acetate	8-hour PEL 15-min STEL	100 ppm (525 mg/m <sup>3</sup> ) none	OSHA, 29CFR1910
1-Pentyl Acetate	MAK Ceiling Value	100 ppm (270 mg/m <sup>3</sup> )	ACGIH, 2002

1: PEL: Permissible Exposure Limit; TWA: Time Weighted Average; MAK: Maximum Concentration

2: 1-pentyl acetate is the major component of Primary Amyl Acetate

Detailed information regarding ventilation and safety equipment used in plants where Primary Amyl Acetate is produced or utilized are not readily available. However, based on the flammable and irritating properties of Primary Amyl Acetate, as well as warnings, handling, and ventilation precautions contained in the material safety data sheet, it is anticipated that vapor concentrations will be maintained at a minimal level and that personal protective equipment will be utilized at locations where over-exposures may occur.

### 2.3.2 Consumer Exposure

A recent database search did not reveal any consumer products in the United States that contain Primary Amyl Acetate as an ingredient (although if used in fragrances, the chemical may not appear in this database). The consumer product categories searched included the following categories: automotive, household, pesticides, landscape/yard, personal care, home maintenance, hobby/crafts, and pet care (NIH, 2004).

It should be noted, however, that OEM automotive touch-up paints can be made available to the consumer; these paints may contain Primary Amyl Acetate although because of its high flashpoint and the need for long shelf life, it is unlikely that Primary Amyl Acetate is contained in these products.

Although not isolated or produced by the sole U.S. manufacturer of Primary Amyl Acetate, 1-pentyl acetate (also known as amyl acetate) is reportedly used in the formulation of consumer products including paints, lacquers, coatings, polishes, photographic films, and cosmetics. In 1988 it was reportedly used as a solvent in nail polish removers in combination with other solvents such as ethyl acetate or acetone (CTFA, 1988)

1-Pentyl acetate, the major component of Primary Amyl Acetate, occurs in the mixture at a concentration of approximately 65%. 1-Pentyl acetate and other unspecified amyl acetates have been detected in a variety of foods including nectarines, apples, kiwi fruit, banana oil, pear oil, yeast, liquors, baked potatoes, and fried chicken (HSDB, 2002; CTFA, 1988; Bisesi, 1994; Sax and Lewis, 1987). The general population may be exposed to the individual components of Primary Amyl Acetate by ingesting food that contains low levels of these materials.

Primary Amyl Acetate is not approved or marketed for use as a direct or indirect food additive. In the United States, 1-pentyl acetate is approved by the Food and Drug Administration (FDA) as an indirect food additive for use only as a component of adhesives in food packaging (21 CFR 175.105). It should be noted, however, that Dow Chemical Company, the sole U.S. manufacturer of Primary Amyl Acetate (mixed isomers), does not isolate and market 1-pentyl acetate.

A database search indicated no consumer products in the United States that list Primary Amyl Acetate as an ingredient in consumer products in the United States.

The concentration of Primary Amyl Acetate in consumer products currently on the market in Western Europe or Japan is not available. In those markets, the general population may be exposed during use of consumer products that contain Primary Amyl Acetate; exposure may be through contact with the skin or by inhalation.

### 2.3.3 Indirect Exposure via the Environment

Human exposure to the components of Primary Amyl Acetate may occur by indirect exposure to air and water with very low concentrations of 1-pentyl acetate and 2-methyl butyl acetate released from both natural and man-made sources. Primary Amyl Acetate may be released to the environment as a fugitive emission during production and use. The individual components of Primary Amyl Acetate may also be released from food products, landfills, and sewage.

1-Pentyl acetate was detected in drinking water in one of 14 plants tested in the United Kingdom (Fielding, et al., 1981). Shackelford et al. (1983) detected relatively high concentrations in effluent



originating from an explosive manufacturing plant (26 ppm) and from a porcelain/enameling plant (31 ppm).

### 3.0 HUMAN HEALTH HAZARDS

#### 3.1 Effects on Human Health

##### 3.1.1 Toxicokinetics, Metabolism and Distribution

###### Studies in Animals

Respiratory bioavailability experiments in rats with Primary Amyl Acetate (65% 1-pentyl acetate, 35% 2-methylbutyl acetate) demonstrated the hydrolysis of these acetate esters to their corresponding alcohols (Poet, 2004a). Rats were exposed to 2000 ppm and blood was withdrawn at regular intervals from an indwelling cannula in the jugular vein. At their peak concentrations (which occurred between 10 and 20 minutes after the start of a 90-minute inhalation exposure), the blood level of 1-pentanol (59 µM) was 2-fold higher than the peak concentration of 1-pentyl acetate (32 µM) and the blood level of 2-methylbutanol (47 µM) was five-fold higher than 2-methylbutyl acetate (10 µM). Blood levels of the corresponding acids (1-pentanoic acid and 2-methylbutyric acid) reached peak levels of 3 µM 1-pentanoic acid and 17 µM 2-methylbutyric acid. The formation of the alcohol isomers during inhalation exposure to Primary Amyl Acetate demonstrates that *in vivo* exposures to Primary Amyl Acetate will lead to appreciable blood levels of the alcohol metabolites.

Inhalation studies in rats with propyl acetate, isopropyl acetate, and isobutyl acetate have demonstrated similar results (Poet, 2003a,b,c). *In vitro* metabolic disposition studies with aliphatic acetate esters have also demonstrated rapid hydrolysis (Dalh, Miller, and Petridou-Fischer, 1987).

###### Studies in humans

In an *in vitro* study using human skin samples, Ward (2000) concluded that both isomers of Primary Amyl Acetate were moderately-well absorbed through the human epidermis.

##### 3.1.2 Acute Toxicity

###### Studies in Animals

###### *Inhalation*

A group of 10 rats (5 male, 5 female) was exposed to Primary Amyl Acetate as a substantially saturated vapor for six hours; average chamber concentrations for males and females were 3693 and 3628 ppm (equivalent to 19,646 and 19,300 mg/m<sup>3</sup>), respectively (Myers, 1983). All animals displayed signs of toxicity which included breathing difficulties, periocular wetness, and immobility; abnormal righting reflex and abnormal toe and tail pinch reflex was noted immediately following exposure. One male died; the only gross pathological finding was redness of the lungs. Surviving rats appeared normal during the 14-day observation interval. In a 4-hour exposure study, male and female rats exposed to 976 ppm (5192 mg/m<sup>3</sup>) for 4 hours appeared normal during and after exposure and during the 14 day observation interval (Myers, 1983).

In an earlier study, there was no mortality among rats exposed to substantially saturated Primary Amyl Acetate vapor (approximately 5200 ppm or 27,776 mg/m<sup>3</sup>) for 4 hours; 100% mortality was observed among rats exposed for 8 hours (Smyth et al., 1962).

An acute neurotoxicity study exposed groups of rats (10 males and 10 females per group) to Primary Amyl Acetate concentrations of 0, 500, 1500, and 3000 ppm (0, 2660, 7980, 15,960 mg/m<sup>3</sup>) for a single 6 hour interval. Animals were observed during exposure. Some differences in body position and eye closure were noted between control and exposed groups which may have been due to the irritating properties of the test material. Rats were subjected to neurotoxicity testing immediately after exposure, and again on Day 1 and Day 8 after exposure. There was no mortality and no treatment-related changes were observed in functional observational battery (FOB) evaluations or automated motor activity assessments. The NOEL for the study for neurotoxicity was 3000 ppm (Beyrouthy, 1997).

In an acute mouse neurotoxicity study, groups of 8 male mice were exposed for 20 minutes to amyl acetate (isomers not specified) at concentrations of 0, 500, 1000, 2000, or 4000 ppm (0, 2660, 5320, 10,640, 21,280 mg/m<sup>3</sup>). There was no mortality and no changes in motor activity were observed. During exposure, mice in the 4000 ppm group displayed palpebral closure and decreased rearing activity. After exposure, mice from the 2000 and 4000 ppm group displayed transient decreased arousal and increased handling reactivity (Bowen and Balster, 1997).

### *Dermal*

Groups of rabbits were exposed for a single 24-hour interval to Primary Amyl Acetate applied full strength under occluded conditions to the clipped skin. The dermal LD<sub>50</sub> for Primary Amyl Acetate in male rabbits was 8359 mg/kg bw; in female rabbits, the LD<sub>50</sub> was >14,080 mg/kg bw (Myers, 1983). Signs of irritation at the site of application of included erythema, ecchymosis, desquamation, and scab formation. Signs of toxicity observed in males included decreased activity and prostration prior to death.

### *Oral*

The oral toxicity of Primary Amyl Acetate was evaluated in groups of male and female rats; the test material was administered undiluted at doses of 0, 4, 8, or 16 ml/kg. A dose of 16 ml/kg bw (14,064 mg/kg bw) killed 2 of 5 rats after one day; the LD<sub>50</sub> in male rats was therefore greater than 16.0 ml/kg bw (14,064 mg/kg bw). The LD<sub>50</sub> in females was 14.0 ml/kg bw or 12,306 mg/kg bw. All survivors recovered within two days. Signs of toxicity included sluggishness, depressed respiration, unsteady gait, lacrimation, and prostration. There was no mortality at the lower doses, and sluggishness was the only sign of toxicity observed (Myers, 1983). An earlier study conducted by Myers (1982) reported an oral LD<sub>50</sub> value in female rats of 19.7 ml/kg bw or 17,250 mg/kg bw for Primary Amyl Acetate, which was administered undiluted.

### Conclusion

Available information suggests that Primary Amyl Acetate (mixture of 2 isomers) is practically non-toxic to animals by oral and dermal contact and is only slightly toxic by inhalation (see Table 5). Primary Amyl Acetate also appears to induce minimal hepatotoxicity, even when high doses are administered by IP injection (Divincenzo and Krasavage, 1974). Although no effect was observed in rats, signs of decreased central nervous system function were observed following high acute

exposures to Primary Amyl Acetate in mice. This effect is commonly observed following exposure to short chain alcohols and esters of these alcohols (Brabec, 1993; Haggard et al., 1945).

Table 5: Summary of Acute Toxicity Studies of Primary Amyl Acetate<sup>1</sup>

Test Material	Species	Sex	Route	Type <sup>2</sup>	Value	Score <sup>3</sup>	Reference
Primary Amyl Acetate	Rat	M/F	Inhalation of vapor, 6 hr	LC50	3693 ppm (19646 mg/m <sup>3</sup> ) killed 1/5 males; 3628 ppm (19300 mg/m <sup>3</sup> ) killed 0/5 females	1	Myers, 1983
Primary Amyl Acetate	Rat	M/F	Inhalation of vapor, 4 hr.	LC50	976 ppm (5192 mg/m <sup>3</sup> ) killed 0/5 males, 0/5 females	1	Myers, 1983
Primary Amyl Acetate	Rat	M	Inhalation of vapor, 4 hr	LC50	5200 ppm (27664 mg/m <sup>3</sup> ) killed 0/6	2	Smyth et al., 1962
Primary Amyl Acetate	Rat	M	Inhalation of vapor, 8 hr	LC50	5200 ppm (27664 mg/m <sup>3</sup> ) killed 6/6	2	Smyth et al., 1962
Primary Amyl Acetate	Rat	M/F	Inhalation of vapor, 6 hr	Other	No mortality at concentrations up to 3000 ppm (15960 mg/m <sup>3</sup> )	2	Beyrouthy, 1997a
Amyl Acetate (isomers not specified)	Mouse	M	Inhalation of vapor, 20 min	Other	No mortality at concentrations up to 4000 ppm (21280 mg/m <sup>3</sup> )	2	Bowen and Balster, 1997
Primary Amyl Acetate	Rabbit	M/F	Dermal	LD50	8359 mg/kg bw (M) >14,080 mg/kg bw (F)	1	Myers, 1983
Primary Amyl Acetate	Rabbit	M	Dermal	LD50	>17,580 mg/kg bw	2	Smyth et al., 1962.
Primary Amyl Acetate	Rat	M/F	Oral	LD50	>14,064 mg/kg bw (M) 12,306 mg/kg bw (F)	1	Myers, 1983
Primary Amyl Acetate	Rat	F	Oral	LD50	17,250 mg/kg bw	2	Myers, 1982
Primary Amyl Acetate	Guinea Pig	M	Intraperitoneal Injection	Other	1500 mg/kg bw killed 3/4	2	Divincenzo and Krasavage, 1974

1: Primary Amyl Acetate is a mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl butyl acetate

2: LD = Lethal Dose; LC = Lethal Concentration

3: Reliability Score

### 3.1.3 Irritation

#### Skin Irritation

##### *Studies in Animals*

Application of 0.5 ml undiluted Primary Amyl Acetate to the clipped intact skin of rabbits for 4 hours under occluded conditions produced moderate irritation with well-defined irritation and slight edema. Seven days after dose, erythema was still evident in two of six animals and desquamation was evident in all animals (Myers, 1983). Application of 0.01 ml to the skin of 5 rabbits under non-occluded conditions for a 24-hour interval resulted in minor irritation, described as Grade 3 on a

Draize scale of 10 (Smyth et al., 1962). These data indicate that under non-occluded conditions, a small volume of Primary Amyl Alcohol on the skin may produce minor irritation.

### Eye Irritation

#### *Studies in Animals*

Instillation of 0.1 ml undiluted Primary Amyl Acetate directly into the conjunctival sac of the rabbit eye resulted in moderate conjunctival irritation in six of six animals, which persisted for as long as 3 days. No corneal injury or iritis was observed (Myers, 1983). Instillation of a flooding volume 0.5 ml of undiluted Primary Amyl Acetate into the rabbit eye resulted in eye irritation and minimal corneal injury described as Grade 2 on a Draize scale of 0 to 10 (Smyth et al., 1962).

These results indicate that Primary Amyl Acetate can cause moderate eye irritation and may cause minor corneal injury.

### Respiratory Tract Irritation

#### *Studies in Animals*

Acute inhalation toxicity studies in rats and mice have been conducted with Primary Amyl Acetate vapor at concentrations up to 5200 ppm. In one study, animals exposed to 3620 ppm (19,258 mg/m<sup>3</sup>) Primary Amyl Acetate vapor exhibited breathing difficulties, which may have been caused by respiratory irritation (Myers, 1983).

In a study with inadequate documentation, Alarie (1981) investigated the irritant effect of inhalation of 1-pentyl acetate on the respiratory rate in mice. There was an approximately 25% decrease in respiratory rate when mice were exposed to 700 ppm; at 2000 ppm, there was an approximately 72% decrease. The RD50 was determined to be 1531 ppm.

#### *Studies in Humans*

Male and female human volunteer subjects were exposed for 3 to 5 minutes to different concentrations of amyl acetate vapour (identity of actual isomer not specified). Following each exposure, subjects classified the degree of irritation of the eyes, nose and throat. Amyl acetate caused mild eye and nose irritation and severe throat irritation at 200 ppm. Slight transient throat discomfort was experienced at 100 ppm (Nelson et al., 1943). Exposure to 900 ppm primary amyl acetate resulted in throat irritation and cough, marked nasal secretion with dryness of the throat, and slight fatigue (Browning, 1965).

### Conclusion

Undiluted Primary Amyl Acetate can produce moderate skin irritation under occluded conditions, and moderate eye irritation with minimal corneal damage. Animals exposed to vapor concentrations of approximately 3600 ppm (19,152 mg/m<sup>3</sup>) exhibited breathing difficulties, probably caused by respiratory irritation. Humans exposed to 100 ppm amyl acetate vapor experienced transient irritation of the throat; moderate irritation of the eyes, nose and throat were reported at concentrations between 200 and 900 ppm. Primary Amyl Acetate is a moderate eye and skin irritant and should also be considered a potential upper respiratory tract irritant.

### 3.1.4 Sensitization

There was no evidence of delayed contact hypersensitivity and no irritation or adverse reactions were observed in a repeat-insult patch test of 20% Primary Amyl Acetate conducted in human test subjects (CTFA, 1987a). Negative results were also obtained in a human photoallergy and primary phototoxicity test on 20% Primary Amyl Acetate (CTFA, 1987b).

Ambiguous results were obtained in a Guinea pig maximization test (Auletta, 1983; Ballantyne and Auletta, 1986). In this study, the test substance was a mixture of three amyl acetates which included 3-methyl butyl acetate as well as the typical components of Primary Amyl Acetate (1-pentyl acetate and 2-methyl butyl acetate). During the induction phase, animals received intradermal injections of Primary Amyl Acetate at concentrations of 100% or 5% (with adjuvant) followed by topical application of the undiluted test material. For the challenge portion of the study, topical application of 100% of the test material was applied for 24 hr. Animals (17/20) challenged with the test material displayed barely perceptible (+/-) erythema, and 4/17 displayed slight edema. Half the control animals displayed +/- erythema. At 48 hr, only 2/20 test animals displayed +/- erythema. The response observed was considered more indicative of transient irritation than sensitization.

### 3.1.5 Repeated Dose Toxicity

#### Studies in Animals

##### *Inhalation*

There is a GLP repeated-dose inhalation study available for Primary Amyl Acetate (mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl butyl acetate). Male and female rats (20 per sex per group) were exposed to Primary Amyl Acetate vapor at concentrations of 0, 100, 300, or 500 ppm (0, 532, 1596, 2660 mg/m<sup>3</sup>). Animals were exposed for 6 hours/day, 5 days/week, for 14 weeks. At the end of the exposure interval, 10 rats per sex per exposure group were sacrificed, and the remainder held for a one-month observation interval. There were no clinical signs of toxicity and no mortality in any of the exposure groups. Among males, all exposure groups displayed similar, decreases in body weight gains of about 10 percent when compared to the concurrent control animals, but not when compared to historical control data. Concurrent control males displayed abnormally large body weight gains that were approximately 13% greater than historical control values. In addition, the inhalation study by Beyrouthy (1997) and the oral study by Weil (1985) did not show changes in body weight. There were no differences for any of the groups in clinical chemistry, hematology, or urinalysis parameters. There were differences in the absolute weight of some organs among exposed animals. However, there was no dose response and no abnormalities were observed upon microscopic examination of organs and tissues. There were no differences in organ weights or body weights in animals at the end of the one-month recovery interval. The NOAEC for this study for male and female rats was 500 ppm or 2660 mg/m<sup>3</sup>, the highest concentration tested (Klonne, 1985).

A 13-week inhalation neurotoxicity study in rats is also available. Groups of male and female rats (15 rats per sex per group) were exposed to Primary Amyl Acetate vapor at concentrations of 0, 300, 600, and 1200 ppm (0, 1596, 3192, 6384 mg/m<sup>3</sup>). Animals were exposed for 6 hours/day, 5 days/week, for 13 weeks. During exposure, animals were observed for overt signs of reaction to the test atmospheres. Exposed animals were examined individually for behavioral changes using automated motor activity measurements and a functional observation battery (FOB) during pre-study and once during weeks 4, 8, and 13. There was no mortality and no signs of toxicity were observed in any group; there were no changes in food consumption or body weight gain. A transient

subtle decrease in activity was noted in the 600 and 1200 ppm group, but only during the first two weeks of exposure. There were no effects observed using motor activity measurements, FOB evaluations, and neuropathological examinations. The NOAEC for repeated-dose neurotoxicity was 1200 ppm or 6384 mg/m<sup>3</sup> (Beyrouty, 1997).

Groups of male and female rats (10 per sex per group) exposed to Primary Amyl Acetate vapor at concentrations of 0, 300, 600, and 1200 ppm (0, 1596, 3192, 6384 mg/m<sup>3</sup>) for 6 hours/day, 5 days a week for 9 days. Animals were observed to have partially closed eyes during exposure; when removed from the exposure chamber, animals appeared normal.

No other signs of toxicity or treatment-related effects were noted in any exposed group with the exception of a slight increase in kidney weights in males. The NOAEC for this study was 600 ppm or 3192 mg/m<sup>3</sup> (Snellings, 1984).

### *Dermal*

Primary Amyl Acetate is a moderately irritating when applied to the skin. There are no repeated-dose dermal toxicity studies for Primary Amyl Acetate or its components, 1-pentyl acetate and 2-methyl butyl acetate.

### *Oral*

A repeat-dose feeding study is available for Primary Amyl Acetate. Groups of 10 male and 10 female rats received doses of Primary Amyl Acetate in their diet at concentrations of 0, 0.1, 0.5, or 1.0% for 90 (males) or 91 (females) days. Doses in males were equivalent to 0, 68, 320, or 650 mg/kg bw/day; female doses were 0, 74, 350, and 720 mg/kg bw/day. There were no abnormalities in appearance or behavior of rats in any of the exposure groups. There were no differences between treated and control animals in body weight, food and water consumption, body weight gain, clinical chemistry and urinalysis parameters, and organ histopathology. The NOAEL for this study for male and female rats was 1% Primary Amyl Acetate in the diet, or 650 mg/kg/day in males and 720 mg/kg bw/day in females, based on quantity of diet consumed (Weil, 1958).

### Conclusion

Primary Amyl Acetate is a moderate irritant to the skin and eyes, and a potential upper respiratory tract irritant. Repeated-dose toxicity studies in male and female rats with Primary Amyl Acetate have demonstrated a low order of toxicity by the inhalation and oral routes of exposure.

Table 6: Repeated-dose Toxicity Studies for Primary Amyl Acetate

Test substance	Species	Dose Levels	Route/ Duration	Lesions Observed	Score <sup>1</sup>	Reference
Primary Amyl Acetate <sup>2</sup>	Rat (M/F)	0, 100, 300, 500 ppm	Inhalation/ 14 weeks	None, no mortality NOAEC = 500 ppm (2660 mg/m <sup>3</sup> )	1	Klonne, 1985
Primary Amyl Acetate	Rat (M/F)	0, 300, 600, 1200 ppm	Inhalation/ 13 weeks	No mortality, no signs of toxicity NOAEC for neurotoxicity = 1200 ppm (6384 mg/m <sup>3</sup> )	1	Beyroudy, 1997
Primary Amyl Acetate	Rat (M/F)	0, 300, 600, 1200 ppm	Inhalation/ 9 days	Increase in kidney weights in males at 1200 ppm; NOAEC = 600 ppm (3192 mg/m <sup>3</sup> )	1	Snellings, 1984
Primary Amyl Acetate	Rat (M/F)	0, 0.1, 0.5, 1.0% in diet	Oral in diet/ 90 days	None, no mortality NOAEL = 1% in diet or 650 mg/kg bw-day for males, 720 mg/kg bw-day for females	2	Weil, 1958

1: Reliability score

2: Primary amyl acetate is composed of 1-pentyl acetate and 2-methyl-1-butyl acetate

### 3.1.6 Genotoxicity

#### Studies in Animals

##### *In Vitro Studies*

**Gene Mutations.** Primary Amyl Acetate was tested in the *Salmonella typhimurium* Mutagenicity Assay (Ames Test), using the standard plate incorporation method (Ames et al., 1975) and tester strains TA 98, TA100, TA1535, TA1537, and TA1538. Primary Amyl Acetate was tested in triplicate at doses of 0, 0.01, 0.03, 0.1, 0.3, and 1.0 µg/plate in the presence and absence of Arochlor-induced liver S-9 mix from Sprague Dawley rats. The highest dose tested was limited by toxicity which was determined by a thinning of the background lawn, and/or a reduction in the number of colonies per plate. Primary Amyl Acetate was not mutagenic when tested to a maximum dose of 1 µg/plate, both in the presence and absence of metabolic activation. A positive response was obtained with positive controls tested both with and without metabolic activation (Hengler and Slesinski, 1983).

Primary Amyl Acetate was not mutagenic when tested *in vitro* in Chinese Hamster Ovary (CHO) cells at multiple doses ranging between 0.02 and 0.30% (v/v) both in the presence and absence of metabolic activation. Mutagenicity was expressed at the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) locus as resistance to 6-thioguanine (TG). Cytotoxicity was defined as less than 10% survival to total lethality. In cultures with acceptable cytotoxicity, Primary Amyl Acetate did not produce an increase in mutation frequency. A positive response was obtained with positive controls tested both with and without metabolic activation (Slesinski, 1983).

**Chromosomal Aberrations.** Primary Amyl Acetate was tested in a GLP *in vitro* chromosomal aberration assay in rat lymphocytes. The study was conducted according to OECD guideline 473, in the presence and absence of rat S-9 metabolic activation. Rat lymphocytes were exposed for 4 or 24 hours to 7 concentrations of the test material ranging between 20.5 and 1310 µg/ml. There was no increase in the frequency of chromosomal aberrations observed in test cultures as compared to

untreated controls. A positive response was obtained with positive controls tested both with and without metabolic activation (Charles et al., 2006).

*Other.* Primary Amyl Acetate was also tested in the *in vitro* Sister Chromatid Exchange (SCE) assay in CHO cells, both in the presence and absence of metabolic activation. CHO cells were exposed to multiple doses ranging between 0.03 and 0.30% (v/v). Significant toxicity was observed at the highest dose tested. In cultures with acceptable levels of toxicity, no significant increase in SCE frequency was observed, both in the presence and absence of metabolic activation. A positive response was obtained with positive controls tested both with and without metabolic activation (Slesinski, 1983).

Table 7: Genotoxicity Studies with Primary Amyl Acetate<sup>1</sup> and 1-Pentyl Acetate<sup>2</sup>

Test substance	Assay	Dose Levels	Metabolic Activation	Results	Score <sup>3</sup>	Reference
<b><i>In Vitro – Bacterial Test Systems</i></b>						
Primary Amyl Acetate	Ames Assay/plate incorporation protocol <i>S. typhimurium</i> tester strains TA98, 100, 1535, 1537, 1538	0, 0.01, 0.03, 0.1, 0.3, 1.0 ug/plate	Rat liver S-9	Negative with and without metabolic activation	2	Hengler and Slesinski, 1983.
<b><i>In Vitro – Non-Bacterial Test Systems</i></b>						
Primary Amyl Acetate	HGPRT Forward Mutation Assay in Chinese hamster ovary (CHO) cells	5 doses up to 0.30% (v/v/)	Rat liver S-9	Negative with and without metabolic activation	2	Slesinski, 1983.
Primary Amyl Acetate	Chromosomal Aberration Assay in rat lymphocytes	0, 20.5, 40.9, 81.9, 163.8, 327.5, 655, and 1310 µg/ml	Rat liver S-9	Negative with and without metabolic activation	1	Charles et al., 2006
Primary Amyl Acetate	Sister Chromatid Exchange (SCE) Cytogenetic Assay in CHO cells	5 doses up to 0.30% (v/v/)	Rat liver S-9	Negative with and without metabolic activation	2	Slesinski, 1983.

1: Primary amyl acetate is composed of 1-pentyl acetate and 2-methyl-1-butyl acetate

2: 1-Pentyl acetate is the major component of Primary Amyl Acetate and is present in the mixture at a concentration of 65%

3: Reliability score

## Conclusion

*In vitro* genotoxicity testing of Primary Amyl Acetate resulted in uniformly negative results (Table 7). *In vitro* studies of Primary Amyl Acetate have been conducted in bacteria as well as animal cell cultures. Primary Amyl Acetate was not mutagenic in the bacteria, *Salmonella typhimurium* (Hengler and Slesinski, 1983) or in CHO cells in the HGPRT forward mutation assay (Slesinski, 1983). Primary Amyl Acetate did not induce an increase chromosomal aberrations in rat lymphocytes (Charles et al., 2006) or in sister chromatid exchanges in CHO cells (Slesinski, 1983).

These results suggest that Primary Amyl Acetate is non-genotoxic *in vitro*.



### 3.1.7 Carcinogenicity

There are no carcinogenicity studies for Primary Amyl Acetate or its components.

### 3.1.8 Reproductive/Developmental Toxicity

#### Studies in Animals

#### *Effects on Fertility*

There are no fertility studies available for Primary Amyl Acetate. Data on the effect of Primary Amyl Acetate on reproductive organs are available from repeated-dose inhalation and feeding studies in male and female rats.

Male and female rats were exposed to 0, 100, 300, or 500 ppm (0, 532, 1596, 2660 mg/m<sup>3</sup>) Primary Amyl Acetate vapor for 6 hr/day, 5 days a week, for 14 weeks. All exposure group males displayed similar, slight decreases in body weight gains and decreases in the absolute weight of the testes. There was no effect on the relative weight of the testes in any group, and no microscopic lesions were observed in the testes. No effect was noted in females, and gross and microscopic evaluations revealed no changes in male or female reproductive organs (Klonne, 1985).

In a feeding study, male and female rats were fed Primary Amyl Acetate in the diet at concentrations of 0, 0.10, 0.5, and 1.0% for 13 weeks. The highest dose level of 1% was equivalent to 650 mg/kg bw/day in males and 720 mg/kg bw/day in females, based on quantity of diet consumed. There was no mortality and no evidence of clinical toxicity was observed. At sacrifice, no abnormalities were observed in male and female gonads between exposed and control animals (Weil, 1958).

Table 8: Summary of Reproductive Toxicity Studies for Primary Amyl Acetate<sup>1</sup>

Test substance	Species	Dose Levels	Route/ Duration	Results	Score <sup>2</sup>	Reference
Primary Amyl Acetate	Rat (M/F)	0, 100, 300, 500 ppm (0, 532, 1596, 2660 mg/m <sup>3</sup> ) 6 hr/day, 5 days/week	Inhalation/ 14 weeks	All doses: decreased BW gain in males, decreased absolute but not relative weight of testes. No other effect on reproductive organ weights, reproductive organs and tissues normal  NOAEC = 500 ppm (2660 mg/m <sup>3</sup> )	1	Klonne, 1985
Primary Amyl Acetate	Rat (M/F)	0, 0.1, 0.5, 1.0% in diet	Oral in diet/ 13 weeks	No effect observed on reproductive organs and tissues  NOAEL = 1% in diet 650 mg/kg bw-day (males) 720 mg/kg bw-day (females)	2	Weil, 1958

1: Primary Amyl Acetate is composed of 1-pentyl acetate and 2-methyl-1-butyl acetate

2: Reliability Score

*Developmental Toxicity*

There are two inhalation developmental toxicity studies (one in rats, one in rabbits) for Primary Amyl Acetate. In a GLP developmental study with Primary Amyl Acetate, groups of 25 pregnant rats were exposed to Primary Amyl Acetate vapor at concentrations of 0, 500, 1000, or 1500 ppm (equivalent to 0, 2660, 5320, 7980 mg/m<sup>3</sup>). Rats were exposed for 6 hours a day during organogenesis (gestational days 6 through 15). Control animals were exposed to air only. Maternal toxicity was observed after exposure ended as evidenced by decreases in food consumption during GD 15-21 and corrected body weight gain. Maternal corrected body weight gain was reduced relative to controls by 13.3% and 20.4% at 1000 and 1500 ppm, respectively. Based on these results, the NOAEC for maternal toxicity is 500 ppm or 2660 mg/m<sup>3</sup>.

There were no effects of exposure on the number of corpora lutea, number of implantations, pre- or post-implantation losses, or sex ratio. The number of fetuses per litter was increased in dams exposed to 1500 ppm.

Fetal examination revealed no significant differences in the incidence of malformations between controls and exposed groups. There was no significant difference in the overall incidence of variations in fetuses exposed to Primary Amyl Acetate. There was an increase in one external (ecchymosis-head) and one visceral (fetal atelectasis) variation in the 1500 ppm group. Female fetal body weights were reduced at 1000 and 1500 ppm. These fetal body weight decreases were accompanied by increases in one or three minor skeletal variations at 1000 and 1500 ppm, respectively. The NOAEC for developmental toxicity in rats was 500 ppm (2660 mg/m<sup>3</sup>). The NOAEC for maternal toxicity is 500 ppm. The NOAEC for developmental toxicity is 500 ppm.

In another GLP developmental study with Primary Amyl Acetate, groups of 15 pregnant rabbits were exposed to Primary Amyl Acetate vapor at concentrations of 0, 500, 1000, or 1500 ppm (0, 2660, 5320, 7980 mg/m<sup>3</sup>). Rabbits were exposed for 6 hours a day during organogenesis (gestational days 6 through 18). Control animals were exposed to air only. Maternal toxicity was observed in the 1500 ppm group as evidenced by reduced food consumption during the exposure interval, and decreases in gestational body weight during gestational day 6 through 12. Body weight gain was reduced in the 1500 ppm group during the entire exposure interval, and corrected body weights were reduced at sacrifice on gestation day 29. There were no effects of exposure on the number of corpora lutea, number of implantations, pre- or post-implantation losses, mean fetal body weights, or sex ratio.

Fetal examination revealed no significant differences in the incidence of malformations between controls and exposed groups. There was no evidence of developmental toxicity or fetotoxicity in any of the exposure groups. There were no exposure-related differences in the incidence of variations or malformations in fetuses exposed to Primary Amyl Acetate relative to controls. The NOAEL for maternal toxicity in rabbits is 1000 ppm or 5320 mg/m<sup>3</sup>; the NOAEL for developmental toxicity is 1500 ppm or 7980 mg/m<sup>3</sup> (Neeper-Bradley and Chun, 1994b).

Table 9: Summary of Developmental Toxicity Studies for Primary Amyl Acetate<sup>1</sup>

Test substance	Species	Dose Levels	Route/ Duration	Results	Score <sup>2</sup>	Reference
Primary Amyl Acetate	Rat (F)	0, 500, 1000, 1500 ppm (0, 2660, 5320, 7980 mg/m <sup>3</sup> )	Inhalation 6 hr/day GD 6-15	Decreased maternal body weight gain and food consumption; reduced body weight of female fetuses; increased incidence of variations; no fetal malformations NOAEC for maternal toxicity: 500 ppm (2660 mg/m <sup>3</sup> ). NOAEC for developmental toxicity: 500ppm (2660 mg/m <sup>3</sup> )	1	Neeper-Bradley and Chun, 1994a
Primary Amyl Acetate	Rabbit (F)	0, 500, 1000, 1500 ppm (0, 2660, 5320, 7980 mg/m <sup>3</sup> )	Inhalation 6 hr/day GD 6-18	Reduced food consumption and decreased maternal body weight; no fetal malformations, no developmental toxicity. NOAEC for maternal toxicity: 1000 ppm (5320 mg/m <sup>3</sup> ). NOAEC for developmental toxicity: 1500 ppm (7980 mg/m <sup>3</sup> )	1	Neeper-Bradley and Chun, 1994b

1: Primary Amyl Acetate is composed of 1-pentyl acetate and 2-methyl-1-butyl acetate

2: Reliability Score

### Conclusion

A GLP compliant repeat-dose inhalation toxicity study, which examined the effect of exposure on reproductive organ weights and histology, has been conducted with Primary Amyl Acetate. There were no significant effects on reproductive organ weights and no lesions were observed upon gross or microscopic examination of male and female reproductive organs. In an earlier 90-day feeding study with Primary Amyl Acetate, reproductive organs were normal after gross and histological examinations. It is anticipated that Primary Amyl Acetate is not a reproductive toxicant, however exposure may produce clinical signs associated with repeated or prolonged contact with a moderately irritating material.

A GLP compliant developmental study in rats was conducted with Primary Amyl Acetate by the inhalation route of exposure. In this study, no malformations were observed, even at concentrations which elicited maternal toxicity. There was no significant increase in the overall number of variations. Female fetal body weights were reduced at 1000 and 1500 ppm. These fetal body weight decreases were accompanied by increases in one or three minor skeletal variations at 1000 and 1500 ppm, respectively. Increases in one external (ecchymosis-head) and one visceral (fetal atelectasis) variation in the 1500 ppm group were also observed. The NOAEC for developmental toxicity in rats was 500 ppm (2660 mg/m<sup>3</sup>).

A GLP-compliant developmental toxicity study in rabbits by the inhalation route of exposure elicited maternal toxicity, but no evidence of developmental toxicity. These results suggest that Primary Amyl Acetate may induce maternal and developmental toxicity but does not induce fetal malformations.

### 3.2 Initial Assessment for Human Health

Primary Amyl Acetate is moderately irritating to the skin and eyes, and is a potential upper respiratory tract irritant. Acute studies have demonstrated that Primary Amyl Acetate has low toxicity by oral and skin exposure, and is only slightly toxic upon vapor inhalation exposure. *In vivo* and *in vitro* genetic toxicity test results suggest that Primary Amyl Acetate should not be considered a genotoxicant. No significant adverse effects were noted in a repeated-dose study in rats exposed to Primary Amyl Acetate vapor by inhalation; similar results were obtained in a 90-day feeding study. Developmental studies in rats and rabbits have demonstrated that Primary Amyl Acetate can cause maternal toxicity but does not produce fetal malformations. In rats, Primary Amyl Acetate can cause reduced fetal body weights and variations at doses that elicit maternal toxicity.

## 4.0 HAZARDS TO THE ENVIRONMENT

There are adequate aquatic toxicity data for Primary Amyl Acetate. Supplemental data for its major component, 1-pentyl acetate are presented in Table 10. In addition, data from the structurally-related chemicals 1-propyl acetate and 1-butyl acetate are also used. Finally, ECOSAR v.0.99g (USEPA, 2000) was used to estimate aquatic toxicity values for the components of Primary Amyl Acetate and their structural analogs (Table 11).

### 4.1 Aquatic Effects

In general, information on the aquatic toxicity of Primary Amyl Acetate is limited to acute studies. Supporting data for its major component, 1-pentyl acetate, and chemicals with similar structures are also presented (Table 10). *Daphnia* appear to be more sensitive to Primary Amyl Acetate than fish. The available data indicate that ecologically significant acute toxicity to aquatic life may occur at concentrations as low as 40 mg/L (Marino et al., 2003).

#### Acute Toxicity Test Results

##### *Fish*

Primary Amyl Acetate was tested in a valid 96-hour static test with the fathead minnow, *Pimephales promelas*, according to Standard Methods for Examination of Water and Wastewater (APHA, 1971). In this study, the 48- and 96-hour LC<sub>50</sub> was 69 mg/L (Waggy and Payne, 1974) based on nominal concentrations.

Valid flow-through and static tests with fish are available for structural analog compounds, 1-propyl acetate and 1-butyl acetate. These tests are included to augment the static, nominal concentration data presented for Primary Amyl Acetate. For propyl acetate, a flow-through and a static test are available for fathead minnows with a range of 96-hr LC<sub>50</sub> values of 60 to 81.7 mg/L. A 96-hr LC<sub>50</sub> value of 18 mg/L for 1-butyl acetate was obtained from a flow-through test with fathead minnows (Brooke et al., 1984).

For Primary Amyl Acetate, the estimated 96-hr LC<sub>50</sub> for fish is 12 mg/L using ECOSAR. For 1-pentyl acetate, the ECOSAR-estimated 96-hr LC<sub>50</sub> for fish is 13 mg/L. For 2-methyl butyl acetate, ECOSAR estimated a 96-hr LC<sub>50</sub> for fish of 14 mg/L,

### *Invertebrates*

There is one GLP study that evaluated the toxicity of Primary Amyl Acetate on *Daphnia magna* according to OECD Guideline 202. The 48-hour EC<sub>50</sub> for this study was 40.9 mg/L, and the NOEC was 3.77 mg/L (Marino et al., 2003). Bringmann and Kuhn (1977) reported a 24-hr EC<sub>50</sub> of 210 mg/L for *Daphnia* in response to 1-pentyl acetate, the major component of Primary Amyl Acetate. The toxicity of Primary Amyl Acetate to freshly hatched brine shrimp (*Artemia salina*) has also been evaluated. The 24-hour LC<sub>50</sub> was determined to be 53 mg/L (Price, Waggy, and Conway, 1974).

For Primary Amyl Acetate, the estimated 48-hr EC<sub>50</sub> in *Daphnia* is 43 mg/L. For 1-pentyl acetate, ECOSAR estimated a 48-hr EC<sub>50</sub> in *Daphnia* of 51 mg/L. Finally, for 2-methyl butyl acetate, ECOSAR estimated a 48-hr EC<sub>50</sub> for *Daphnia* of 60 mg/L.

### *Algae*

There is one GLP study that evaluated the effect of Primary Amyl Acetate on the green algae *Pseudokirchnerilla subcapitata*. The 72-hr and 96-hr EC<sub>50</sub>s (based on growth rate) were determined to be >466 mg/L. Based on biomass, the 72-hr EC<sub>50</sub> was 156 mg/L and the 96-hr EC<sub>50</sub> was 235 mg/L. Based on cell density, the 72-hr EC<sub>50</sub> was 242 mg/L; the 96-hr EC<sub>50</sub> was 313 mg/L (Hancock et al., 2003). NOEC values based on cell density could not be determined; however, the 96-hr EC<sub>10</sub> values were determined to be 26.3 mg/L.

For Primary Amyl Acetate, the estimated 96-hr EC<sub>50</sub> for green algae is 1.0 mg/L. For 1-pentyl acetate, ECOSAR estimated a 96-hr EC<sub>50</sub> for green algae of 1.0 mg/L. Finally, for 2-methyl butyl acetate, ECOSAR estimated a 96-hr LC<sub>50</sub> for green algae of 1.2 mg/L.

### *Microorganisms*

There are limited data for 1-pentyl acetate, the major component of Primary Amyl Acetate. In the bacteria, *Escherichia coli* and *Pseudomonas putida*, the 3% reduction in growth relative to control cultures (EC<sub>03</sub>) after an exposure interval of 16 hours was determined to be 145 mg/L for both species (Bringmann and Kuhn, 1980). In the protozoa *Entosiphon sulcatum*, the 72-hour EC<sub>03</sub> was 226 mg/L (Bringmann and Kuhn, 1980).

Table 10: Summary of Acute Environmental Toxicity Data for Primary Amyl Acetate and Structural Analogs

Test Substance	Test Organism	LC <sub>50</sub> or EC <sub>50</sub> (mg/L)	Duration/Endpoint	Score <sup>1</sup>	Reference
<b>Acute - Fish</b>					
Primary Amyl Acetate <sup>2</sup>	Fathead minnow <i>Pimephales promelas</i>	48-hr LC <sub>50</sub> = 69 96-hr LC <sub>50</sub> = 69	96 hr static; mortality	2	Waggy and Payne, 1974.
1-Propyl Acetate <sup>3</sup>	Fathead minnow <i>Pimephales promelas</i>	96-hr LC <sub>50</sub> = 60	96 hr flow thru; mortality	1	Brooke et al., 1984
1-Propyl Acetate	Fathead minnow <i>Pimephales promelas</i>	96-hr LC <sub>50</sub> = 81.7	96 hr static; mortality	2	Waggy and Payne, 1974
1-Butyl Acetate <sup>4</sup>	Fathead minnow <i>Pimephales promelas</i>	96-hr LC <sub>50</sub> = 18	96 hr flow thru; mortality	1	Brooke et al., 1984
<b>Acute - Invertebrates</b>					
Primary Amyl Acetate	Water flea <i>Daphnia magna</i>	48-hr EC <sub>50</sub> = 40.9 48-hr NOEC = 3.77	48 hr static; immobilization	1	Marino et al., 2003
Primary Amyl Acetate	Brine shrimp <i>Artemia salina</i>	24-hr LC <sub>50</sub> = 53	24 hr static; mortality	2	Price, Waggy, and Payne, 1974
<b>Acute - Algae</b>					
Primary Amyl Acetate	Green algae <i>Pseudokirchnerilla subcapitata</i>	72-hr E <sub>b</sub> C <sub>50</sub> = 156 96-hr E <sub>b</sub> C <sub>50</sub> = 235 72-hr E <sub>r</sub> C <sub>50</sub> = >466 96-hr E <sub>r</sub> C <sub>50</sub> = >466	96 hr static; biomass/ biomass growth rate growth rate	1	Hancock et al., 2003
<b>Acute - Microorganisms</b>					
1-Pentyl Acetate <sup>5</sup>	Bacteria <i>Escherichia coli</i>	16-hr EC <sub>03</sub> = 145	16-hr static; reduced growth	4	Bringmann and Kuhn, 1980
1-Pentyl Acetate	Bacteria <i>Pseudomonas putida</i>	16-hr EC <sub>03</sub> = 145	16-hr static; reduced growth	4	Bringmann and Kuhn, 1980
1-Pentyl Acetate	Protozoa <i>Entosiphon sulcatum</i>	72-hr EC <sub>03</sub> = 226	72-hr static; reduced growth	4	Bringmann and Kuhn, 1980

1: Reliability Score: for studies with a score of 1, analytical data for test concentrations administered are available; for studies with a score of 2 or 4, nominal data are available.

2: Primary Amyl Acetate is a mixture of 1-pentyl acetate and 2-methyl-1-butyl acetate

3: 1-Propyl acetate is the 3-carbon structural analog of 1-pentyl acetate, the major component of Primary Amyl Acetate

4: 1-Butyl acetate is the 4-carbon structural analog of 1-pentyl acetate, the major component of Primary Amyl Acetate

5: 1-Pentyl acetate is the major component of Primary Amyl Acetate and is present in the mixture at a concentration of 65%

6: Tests conducted by Bringmann and Kuhn have reliability score of 4 due to non-standard toxicity criteria.

Table 11: Summary of Calculated Acute Environmental Toxicity Values for Components of Primary Amyl Acetate<sup>1</sup> and Structural Analogs

Test Substance	Test Organism	LC <sub>50</sub> or EC <sub>50</sub> (mg/L)	Duration/Endpoint	Reference
<b>Acute - Fish</b>				
1-Propyl Acetate <sup>2</sup>	Freshwater fish	96-hr LC <sub>50</sub> = 39.9	ECOSAR estimated value*	ECOSAR, Version 0.99g
1-Butyl Acetate <sup>3</sup>	Freshwater fish	96-hr LC <sub>50</sub> = 22.0	ECOSAR estimated value	ECOSAR, Version 0.99g
1-Pentyl Acetate <sup>4</sup>	Freshwater fish	96-hr LC <sub>50</sub> = 12.9	ECOSAR estimated value	ECOSAR, Version 0.99g
2-Methyl butyl Acetate <sup>5</sup>	Freshwater fish	96-hr LC <sub>50</sub> = 14.3	ECOSAR estimated value	ECOSAR, Version 0.99g
<b>Acute - Invertebrates</b>				
1-Propyl Acetate	Invertebrate ( <i>Daphnia</i> )	48-hr EC <sub>50</sub> = 407.2	ECOSAR estimated	ECOSAR, Version

Test Substance	Test Organism	LC <sub>50</sub> or EC <sub>50</sub> (mg/L)	Duration/ Endpoint	Reference
			value	0.99g
1-Butyl Acetate	Invertebrate ( <i>Daphnia</i> )	48-hr EC <sub>50</sub> = 134.5	ECOSAR estimated value	ECOSAR, Version 0.99g
1-Pentyl acetate	Invertebrate ( <i>Daphnia</i> )	48-hr EC <sub>50</sub> = 50.7	ECOSAR estimated value	ECOSAR, Version 0.99g
2-Methyl butyl acetate	Invertebrate ( <i>Daphnia</i> )	48-hr EC <sub>50</sub> = 59.9	ECOSAR estimated value	ECOSAR, Version 0.99g
<b>Acute - Algae</b>				
1-Propyl Acetate	Green Algae	96-hr EC <sub>50</sub> = 3.1	ECOSAR estimated value	ECOSAR, Version 0.99g
1-Butyl Acetate <sup>3</sup>	Green Algae	96-hr EC <sub>50</sub> = 1.7	ECOSAR estimated value	ECOSAR, Version 0.99g
1-Pentyl acetate	Green Algae	96-hr EC <sub>50</sub> = 1.0	ECOSAR estimated value	ECOSAR, Version 0.99g
2-Methyl butyl acetate	Green Algae	96-hr EC <sub>50</sub> = 1.1	ECOSAR estimated value	ECOSAR, Version 0.99g

\* ECOSAR values have been assigned a reliability score of 2: valid with restriction, accepted calculation method. The SAR for neutral organics was used; the structure determined from the CAS registry number as stored in the accompanying database of SMILES notations within ECOSAR. The preferred values for water solubility and Log Kow were also utilized.

1: Primary Amyl Acetate is a mixture of 1-pentyl acetate and 2-methyl-1-butyl acetate

2: 1-Propyl acetate is the 3-carbon structural analog of 1-pentyl acetate, the major component of Primary Amyl Acetate

3: 1-Butyl acetate is the 4-carbon structural analog of 1-pentyl acetate, the major component of Primary Amyl Acetate

4: 1-Pentyl acetate is the major component of Primary Amyl Acetate and is present in the mixture at a concentration of 65%

5: 2-Methyl-1-butyl acetate is the minor component of Primary Amyl Acetate and is present at a concentration of 35%

## 4.2 Terrestrial Effects

### Acute Toxicity Test Results

No ecotoxicological data for Primary Amyl Acetate or its components were identified for terrestrial wildlife (*i.e.*, birds and mammals) or other terrestrial organisms (plants, invertebrates, and bacteria).

## 4.3 Other Environmental Effects

No additional data available.

## 4.4 Initial Assessment for the Environment

The available physicochemical data are adequate to describe the properties of Primary Amyl Acetate, mixed isomers. Primary amyl acetate has a vapor pressure of 5.73 hPa at 25 °C, a water solubility of 1700 mg/l at 25 °C; and a measured log K<sub>ow</sub> value of 2.42. The photochemical removal of its components, 1-pentyl acetate and 2-methyl butyl acetate, as mediated by hydroxyl radicals, occurs with calculated half-lives of 34 to 43 hours, respectively. Primary Amyl Acetate is biodegradable under aerobic conditions. Primary Amyl Acetate is anticipated to volatilise easily from moving rivers, but only moderately from quiescent lakes and other surface water bodies (calculated volatilization half-lives for Primary Amyl Acetate of 3.4 hours from a river and 5.5 days from a lake). Primary Amyl Acetate is not likely to bioaccumulate in food webs. Based on Level III distribution modelling, it is estimated that the majority of Primary Amyl Acetate released to the environment will partition into water (26.7%) and soil (66.3%), with a smaller amount in air (6.8%) and sediment (0.14%). The stability of Primary Amyl Acetate in water is pH dependent. Based on

OECD Guideline 111 hydrolysis studies, the predicted half-life values for Primary Amyl Acetate at 25 °C at pH 4, 7, and 9 are 84.8, 138, and 21.9 days, respectively.

Valid aquatic toxicity data (fish, daphnia, and algae) are available for Primary Amyl Acetate. Primary Amyl Acetate was tested in a valid 96-hour static test with the fathead minnow, the 96-hour LC<sub>50</sub> was 69 mg/L. In a valid OECD guideline test with *Daphnia*, the 48-hour EC<sub>50</sub> was 40.9 mg/L, the NOEC was 3.77 mg/L. In a valid OECD guideline test with green algae, the 72-hr EC<sub>50</sub> based on growth rate is >466 mg/L and biomass is 156 mg/L.

Supporting data for analog compounds are also presented for fish to support nominal concentration data available in the fathead minnow study for Primary Amyl Acetate. For propyl acetate, two studies with fathead minnows are available, with 96-h LC50s of 60 to 81.7 mg/L reported. For butyl acetate, a flow-through study with fathead minnows reported a 96-h LC50 of 18 mg/L.

Estimated values using ECOSAR have been determined for the components of Primary Amyl Acetate and surrogate chemicals for acute fish toxicity. Terrestrial data are limited.

## 5.0 RECOMMENDATIONS

These recommendations are applicable only to Primary Amyl Acetate-Mixed Isomers (reaction process-derived product) and not to its individual components.

For human health, the product is currently a low priority for further work. The product possesses properties indicating a hazard for human health (skin, eye and respiratory tract irritation, and potential developmental toxicity). Based on data provided by the sponsor country (relating to production by one producer in the United States which account for an unknown fraction of the global production and relating to the use pattern primarily in the United States), risk management measures are being applied (engineering controls, occupational standards, Material Safety Data Sheets) in occupational settings. Countries may desire to check their own risk management measures for this product to find out whether there is need for additional measures.

For the environment, the product has properties indicating a hazard for the environment (acute aquatic EC/LC50 values between 1 and 100 mg/l). However the product is of low priority for further work for the environment because of its rapid biodegradation and its limited potential for bioaccumulation.



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**ROBUST SUMMARIES****and****SIDS DOSSIER for:****PRIMARY AMYL ACETATE**

(commercial mixture of 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate)

**CAS No.****628-63-7 (1-pentyl acetate) and  
624-41-9 (2-methyl-1-butyl acetate)**

Sponsor Country: U.S.A.

DATE: July 2006



**1.0 GENERAL INFORMATION**

Remark: Data are presented for Primary amyl acetate, a commercial reaction process-derived mixture of two isomers, 1-pentyl acetate and 2-methyl-1-butyl acetate. The ratio of these two isomers in the commercial mixture currently being produced in the Sponsor Country is approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate. Additional data for the individual components are presented in support of data for the mixture.

**1.01 SUBSTANCE INFORMATION**

<b>A. CAS-Number</b>	628-63-7 (1-pentyl acetate) 624-41-9 (2-methyl-1-butyl acetate)
<b>B. Name (IUPAC name)</b>	Acetic acid, pentyl ester
<b>C. Name (OECD name)</b>	N/A, mixed isomers
<b>D. CAS Descriptor</b>	Not applicable in this case
<b>E. EINECS-Number</b>	211-047-3 (1-pentyl acetate) 210-843-8 (2-methyl-1-butyl acetate)
<b>F. Molecular Formula</b>	C7 H14 O2
<b>G. Structural Formula</b>	CH3-COO-CH2-CH2-CH2-CH2-CH3 (1-pentyl acetate) CH3-COO-CH2-CH-(CH3)-CH2-CH3 (2-methyl butyl acetate)
<b>H. Substance Group</b>	Not applicable
<b>I. Substance Remark</b>	None
<b>J. Molecular Weight</b>	130.19

**1.02 OECD INFORMATION**

<b>A. Sponsor Country:</b>	U.S.A.
<b>B. Lead Organisation:</b>	
<b>Name of Lead Organisation:</b>	American Chemistry Council
<b>Contact person:</b>	Barbara Francis
<b>Address:</b>	1300 Wilson Blvd. Arlington, VA 22209 U.S.A. Tel: 703-741-5609 Fax: 703-741-6091

**1.1 GENERAL SUBSTANCE INFORMATION**

- A. Type of Substance** element [ ]; inorganic [ ]; natural substance [ ];  
organic [ X ];  
organometallic [ ]; petroleum product [ ]
- B. Physical State** (at 20°C and 1.013 hPa)  
gaseous [ ]; liquid [ X ]; solid [ ]
- C. Purity** (indicate the percentage by weight/weight)  
>98% weight/weight  
Product supplied as commercial reaction process-derived mixture of 65% 1-pentyl acetate and 35% 2-methyl butyl acetate.

**1.2 SYNONYMS**

(for 1-pentyl acetate)  
acetic acid, amyl ester  
acetic acid, n-amyl ester  
1-amyl acetate  
n-amyl acetate  
primary amyl acetate  
acetic acid pentyl ester  
amyl acetic ester  
1-pentanol acetate  
n-pentyl acetate  
n-pentyl ethanoate  
banana oil

(for 2-methyl-1-butyl acetate)  
2-methyl butyl acetate  
2-methylbutyl acetate  
1-butanol, 2 methyl, acetate  
2-methylbutyl ethanoate  
2-methyl-1-butanol acetate

**1.3 IMPURITIES**

CAS No: 71-41-0  
EINECS No: 200-752-1  
Name: 1-pentyl alcohol  
Value: 0.26%

Remarks:  
Reference:

Cosmetic, Toiletry and Fragrance Association (CTFA). 1986. Analysis of Union Carbide primary Amyl Acetate. Available for review: Director, Cosmetic Ingredient Review, 1110 Vermont Ave., N.W. Suite 810, Washington, DC. *cited in:* Elder, R.L. (ed). 1988. Safety Assessment of Cosmetic Ingredients. Final Report on the Safety Assessment of Amyl Acetate and Isoamyl acetate. J. American College of Toxicology 7: 705-719.

CAS No: 137-32-6  
EINECS No: 205-289-9  
Name: 2-methyl-1-butyl alcohol

Value: 0.20%  
Remarks:  
Reference: CTFA, 1986.

CAS No:  
EINECS No:  
Name: 2-methyl butyl formate  
Value: 0.12%  
Remarks:  
Reference: CTFA, 1986.

CAS No: 123-92-2  
EINECS No: 204-662-3  
Name: Isoamyl acetate  
Value: <0.1%  
Remarks:  
Reference: CTFA, 1986.

#### 1.4 ADDITIVES

CAS No: None  
EINECS No:  
Name:  
Value:  
Remarks: Inhibitors and stabilisers are not applicable. Primary amyl acetate is stable and hazardous polymerisation will not occur.  
Reference: The Dow Chemical Company. Material Safety Data Sheet for Primary Amyl Acetate Mixed Isomers. MSDS# 526, effective date 06/12/2001. The Dow Chemical Company, Midland, MI.

#### 1.5 QUANTITY

9.3 metric tons  
Remarks: consumption in 2001  
Reference: Bizzari, S.n, Gubler, R. and Kishi, A. 2002. CEH Marketing Research Report. Oxo Chemicals.

#### 1.6 LABELLING AND CLASSIFICATION

##### Labelling

Type:

Specific limits:

Symbols:

Note:

R-phrases: R-10: Flammable

R-66: Repeated exposure may cause skin dryness or cracking

S-phrases: S-2: Keep out of reach of children

S-23: Do not breathe gas/fumes/vapour/spray

S-25: Avoid contact with eyes

Remarks:

Classification

Type:

Category of danger:

R-phrases:

Remarks:

## 1.7 USE PATTERN

### A. General

**Type of Use:** **Category: Non dispersive**

Use resulting in inclusion into or onto matrix

<b>Type of Use:</b>	<b>Category: Wide dispersive</b>
industrial	Basic industry: basic chemicals
industrial	Pharmaceutical industry, extraction of penicillin
industrial	Chemical industry: solvent for lacquers, paints
industrial	Solvent for phosphors in fluorescent lamps
use	Manufacture of artificial fruit flavouring agents

Reference: HSDB (Hazardous Substance Databank ). 2002. n-Amyl Acetate (CAS 628-63-7). HSDB No. 5126. Complete update on 07/22/2002.

Clayton, G.D., F.E. Clayton (eds.) Patty's Industrial Hygiene and Toxicology. Volumes 2A, 2B, 2C, 2D, 2E, 2F: Toxicology. 4th ed. New York, NY: John Wiley & Sons Inc., 1993-1994. 298

Lewis, R.L., Sr. 1993. Hawley's Condensed Chemical Dictionary, 12th ed. New York: Van Nostrand Reinhold.

### B. Uses in Consumer Products

Primary Amyl Acetate is also used as a component in OEM (original equipment manufacturer) automotive paints that are available to the consumer. 1-Pentyl acetate, the major component of Primary Amyl Acetate, may be used as a direct solvent for manufacture of paints, lacquers, , fabrics, as a fragrance enhancer in cosmetics..

## 1.8 OCCUPATIONAL EXPOSURE LIMIT VALUE

### A. Values for 1-Pentyl Acetate (CAS 628-63-7)

Type of Limit:	TLV-TWA (US)
Test material:	1-pentyl acetate (628-63-7)

## 1. GENERAL INFORMATION

ID: 628-63-7, 624-41-9

DATE: 01.07.2006

Value:	50 ppm (266 mg/m <sup>3</sup> )
Time Schedule:	Time-weighted-average exposure concentration for a conventional 8-hour workday, and 40-hour workweek.
Short Term Exposure	
Limit Value:	100 ppm (532 mg/m <sup>3</sup> )
Time Schedule:	15-minute time-weighted-average exposure that should not be exceeded any time during a workday
Remark:	8-hr TWA for 1-pentyl acetate TWA: 50 ppm STEL: 100 ppm
Reference:	American Conference of Governmental Industrial Hygienists (ACGIH). 2002. Guide to Occupational Exposure Values-2002. ACGIH, Inc., Cincinnati, OH.
Type of Limit other:	OSHA PEL (US)
Test material:	1-pentyl acetate (628-63-7)
Value:	100 ppm (525 mg/m <sup>3</sup> )
Short Term Exposure	
Limit Value:	None
Time Schedule:	Time-weighted-average exposure concentration for a conventional 8-hour workday, and 40-hour workweek.
Remark:	8-hr Permissible Exposure Limit (TWA) for 1-pentyl acetate TWA: 100 ppm STEL: None
Reference:	American Conference of Governmental Industrial Hygienists (ACGIH). 2002. Guide to Occupational Exposure Values-2002. ACGIH, Inc., Cincinnati, OH.
Type of Limit:	NIOSH REL (US)
Test material:	1-pentyl acetate (628-63-7)
Value:	100 ppm (525 mg/m <sup>3</sup> )
Short Term Exposure	
Limit Value:	None
Time Schedule:	
Remark:	Recommended Exposure Limit (TWA) for 1-pentyl acetate: 100 ppm
Reference:	American Conference of Governmental Industrial Hygienists (ACGIH). 2002. Guide to Occupational Exposure Values-2002. ACGIH, Inc., Cincinnati, OH.
Type of Limit:	MAK (DFG), Ceiling Value
Test substance:	1-pentyl acetate (628-63-7)
Remark:	Ceiling value (concentration shall not be exceeded during any part of the work exposure)
Value:	100 ppm (270 mg/m <sup>3</sup> )
Time Schedule:	15 minutes number allowed per shift: 4 interval between exposures: 1 hour

## 1. GENERAL INFORMATION

ID: 628-63-7, 624-41-9

DATE: 01.07.2006

## Short Term Exposure

Limit Value: Ceiling value, see above

Remark: MAK Category I substance: local irritant effects determine the MAK value

Reference: American Conference of Governmental Industrial Hygienists (ACGIH). 2002. Guide to Occupational Exposure Values-2002. ACGIH, Inc., Cincinnati, OH.

Type of Limit: Dow Chemical Company/Union Carbide Corporation exposure limit

Test substance: 1-pentyl acetate (628-63-7)

Value: 50 ppm (266 mg/m<sup>3</sup>)

## Short Term Exposure

Limit Value: none

Time Schedule: 8 hr time weighted average (TWA)

Reference: The Dow Chemical Company. Material Safety Data Sheet for Primary Amyl Acetate Mixed Isomers. MSDS# 526, effective date 06/12/2001.

The Dow Chemical Company, Midland, MI.

**B. Values for 2-Methyl-1-Butyl Acetate (CAS 624-41-9)**

Type of Limit: TLV-TWA (US)

Test substance: 2-methyl-1-butyl acetate

Value: 50 ppm (266 mg/m<sup>3</sup>)

Time Schedule: Time-weighted-average exposure concentration for a conventional 8-hour workday, and 40-hour workweek.

## Short Term Exposure

Limit Value: 100 ppm (532 mg/m<sup>3</sup>)

Time Schedule: 15-minute time-weighted-average exposure that should not be exceeded any time during a workday

Remark: ACGIH, 8-hr TWA for pentyl acetate, all isomers

TLV: 50 ppm

STEL: 100 ppm

Reference: American Conference of Governmental Industrial Hygienists (ACGIH). 2002. Guide to Occupational Exposure Values-2002. ACGIH, Inc., Cincinnati, OH.

**1.9 SOURCES OF EXPOSURE**

Remark: The majority of information for amyl acetates, when the isomer(s) are not specified, pertain to 1-pentyl acetate (also known as n-amyl acetate or amyl acetate). A database search revealed no consumer products in the United States that contain Primary Amyl Acetate as an ingredient. The consumer products searched included the following categories: automotive, household, pesticides, landscape/yard, personal care, home maintenance, hobby/crafts, and pet care. Primary Amyl Acetate is used, however, as a component in OEM (original equipment manufacturers) automotive paints, which can be obtained by consumers. If the chemical is found in fragrances, this will not necessarily be found within the database.

## 1. GENERAL INFORMATION

ID: 628-63-7, 624-41-9

DATE: 01.07.2006

- Reference: National Institute of Health (NIH). 2004. Ingredients in Consumer Products. National Library of Medicine. Special Information Services. Bethesda, MD. Last update on May 12, 2004.
- Remark: Primary amyl acetate may be used as a fragrance enhancer, typically at low ppm concentrations, in cosmetics.
- Reference: The Dow Chemical Company, 2006
- Remark:: In the past, primary amyl acetate was used in cosmetics, primarily as a solvent for nitrocellulose in nail base coats, and nail polishes, enamels, and lacquers; it also functioned as a solvent in nail polish removers. In enamel removers, amyl acetates may have been used in combination with other solvents such as acetone or ethyl acetate. These uses for Primary Amyl Acetate have been replaced by other solvents.
- Reference: Elder, R.L. (ed). 1988. Safety Assessment of Cosmetic Ingredients. Final Report on the Safety Assessment of Amyl Acetate and Isoamyl acetate. J. American College of Toxicology 7: 705-719.
- Remark: Data submitted to the U.S. Food and Drug Administration in 1987 indicated that 18 cosmetic products contained Primary Amyl Acetate or its major component, 1-pentyl acetate. A recent database search revealed 11 cosmetic products that contained 1-pentyl acetate; no cosmetic products contained Primary Amyl Acetate.
- Reference: U.S. Food and Drug Administration (U.S. FDA). 1987. Cosmetic product formulation data: Ingredients used in each product category. Washington, D.C. National Institute of Health (NIH). 2004. Ingredients in Consumer Products. National Library of Medicine. Special Information Services. Bethesda, MD. Last update on May 12, 2004.
- Remark: Primary amyl acetate is not approved as a natural or synthetic food flavoring substance.
- Reference: Code of Federal Regulations. Volume 21, Part 175.105. Adhesives, Subpart B: Substances for use only as components of adhesives.
- Remark: The major component of Primary Amyl Acetate, 1-pentyl acetate, occurs naturally in fruit, such as volatile aroma of banana oil and pear oil; 1-pentyl acetate has also been identified in yeasts, liquors and is a component of the alarm/defense system of the honey bee.
- Reference: Bisesi, M.S. 1994. Esters. *In*: Clayton, G.D., and Clayton, F.E. (eds.) Patty's Industrial Hygiene and Toxicology. 4th ed, Vol. II D, New York, NY: John Wiley & Sons Inc., p 2985.  
Sax, I.N. and Lewis, R.J., Sr. (eds). Hawley's Condensed Chemical Dictionary, 11<sup>th</sup> Edition. New York, NY: Van Nostrand Reinhold Company, 1987. 74.
- Remark: Occurrence in food may lead to its release to the environment. Amyl acetate occurs naturally in fruits and may be released as a plant volatile. The general population may be exposed to amyl acetate through consumption of food products.  
1-Pentyl acetate was identified but not quantified in nectarines, apples, kiwi fruit, and as a volatile component of baked potatoes and fried chicken.  
Occupational exposure may occur through inhalation or dermal contact with this compound at workplaces where amyl acetate is produced or used as a solvent.
- Reference: HSDB (Hazardous Substance Databank ). 2002. n-Amyl Acetate (CAS 628-63-7). HSDB No. 5126. Complete update on 07/22/2002.

## 1. GENERAL INFORMATION

ID: 628-63-7, 624-41-9

DATE: 01.07.2006

Remark: In a survey of drinking water in the United Kingdom, 1-pentyl acetate was detected in drinking water in one of 14 plants tested; the source of water for this plant was groundwater.

Reference: HSDB (Hazardous Substance Databank ). 2002. n-Amyl Acetate (CAS 628-63-7). HSDB No. 5126. Complete update on 07/22/2002.

**1.10 ADDITIONAL REMARKS**

Remark: NFPA Hazard Classifications:

Flammability: 3 (easily ignited under almost all normal conditions)

Reactivity: 0 (normally stable even under fire exposure conditions)

Health: 1 (exposure would cause irritation but only minor residual injury)

Reference: NFPA. 2002. National Fire Protection Association. Fire Protection Guide to Hazardous Materials, 13th edition. NFPA. Quincy, MA.

Remark: Disposal: Amyl acetate is a waste chemical stream constituent which may be subjected to ultimate disposal by controlled incineration.

Reference: USEPA. 1981. Engineering Handbook for Hazardous Waste Incineration. EPA 68-03-3025. 2-4.

Remark: USDOT/UN/NA/IMO number: 1104

IMO 3.3, 3.2

Standard transportation number: 49 091 11

class: 3

label: 3

pack. gr.: 3

Marine pollutant:

Label: Flammable liquid

Proper shipping name: Amyl acetates

Reference: HSDB (Hazardous Substance Databank ). 2002. n-Amyl Acetate (CAS 628-63-7). HSDB No. 5126. Complete update on 07/22/2002.



## 2.0 PHYSICAL/CHEMICAL PROPERTIES

### 2.1 MELTING POINT

(a) Critical study and preferred value (for the mixture)

Test material: Primary Amyl Acetate, purity 99.52%; mixture of 60.46% 1-pentyl acetate and 39.06% 2-methyl butyl acetate

Value: -94 °C

Remark: The freezing point of Primary Amyl Alcohol was estimated from a simulated 45° eutectic line downward from the preferred freezing point of the 1-pentyl alcohol, the major component of the mixture.

Reliability: score = 2, reliable with restriction; accepted calculation method.

Reference: Olson, J., Wilson, L. and Morrison, R. 2006. Physical Properties Measurements on Amyl Series Chemicals: Amyl Alcohol, Amyl Acetate, and Isopentanoic Acid. Report Code SC-AL.SC-2006-000002, dated January 4, 2006.

(b) Critical study and preferred result (for major component only)

Test material: 1-pentyl acetate

Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate and is present in the mixture at a concentration of approximately 65%

Value: -70.8° C

Remark : -159.4° F

Reliability: score = 2, reliable with restriction; data from Handbook or collection of data.

Reference: Sax, I.N. and Lewis, R.J., Sr. (eds). Hawley's Condensed Chemical Dictionary, 11<sup>th</sup> Edition. New York, NY: Van Nostrand Reinhold Company, 1987. 74.

Lide, D.R. (ed). 1995. CRC Handbook of Chemistry and Physics, 75<sup>th</sup> ed. Boca Raton, FL: CRC Press Inc., 1995-1996.

Hawley, G.G. (ed.). 1982. The Condensed Chemical Dictionary, 8<sup>th</sup> Edition. New York: Van Nostrand Reinhold, p. 58.

Bisesi, M.S. 1994. Esters. *In*: Clayton, G.D., and Clayton, F.E. (eds.) Patty's Industrial Hygiene and Toxicology. 4th ed, Vol. II D, New York, NY: John Wiley & Sons Inc., p 2978.

(c) Test material: 1-pentyl acetate

Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate and is present in the mixture at a concentration of approximately 65%

Value: -78.5° C

Remark : -173.3° F

Reliability: score = 2, reliable with restriction; data from Handbook or collection of data.

Reference: Sax, I.N. 1979. Dangerous Properties of Industrial Materials, 5<sup>th</sup> Edition. New York: Van Nostrand Reinhold. p.373.

- (d) Critical study and preferred result (for minor component only)
- Test material: 2-methyl-1-butyl acetate (CAS # 624-41-9)
- Remark: 2-methyl-1-butyl acetate is the minor component of Primary Amyl Acetate, and is present in the mixture at a concentration of 35%
- Value: -56 °C
- Remark: -100.8 °F
- Reliability: Score=2, valid with restriction; accepted calculated method
- Reference: U.S. EPA. 2003. EPI Suite Software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>

## 2.2 BOILING POINT

- (a) Critical study and preferred result (for the mixture)
- Test material: Primary Amyl Acetate, purity 99.52%; mixture of 60.46% 1-pentyl acetate and 39.06% 2-methyl butyl acetate
- Value: 146.28° C
- Remark: 295.31° F
- Method: ASTM E1719: Standard Test Method for Vapor Pressure of Liquids by Ebulliometry. Annual Book of ASTM Standards, Vol. 14.02.
- Results: Pressures were measured and controlled with a calibrated Mensor Model PCS 400 manostat to +/- 13 Pa. Temperatures were measured with a calibrated Omega platinum resistance thermometer to 0.01 +/- K. Results are presented below in tabular form

Boiling Point Determination for Primary Amyl Acetate

Pressure in mm Hg	Pressure in KPa	Temperature (°C)
10	1.333	38.58
50	6.666	69.66
100	13.332	85.73
300	39.997	115.59
760*	101.325	146.28

\*boiling point

- Year: 2004
- Reliability: Score = 1, valid without restriction, meets national standard methods.
- Reference: Olson, J., Wilson, L. and Morrison, R. 2006. Physical Properties Measurements on Amyl Series Chemicals: Amyl Alcohol, Amyl Acetate, and Isopentanoic Acid. Report Code SC-AL.SC-2006-000002, dated January 4, 2006.
- (b) Critical study and preferred result (for major component only)
- Test material: 1-pentyl acetate (CAS 628-63-7)
- Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%

- |  |              |  |
|--|--------------|--|
|  | Value:       | 148.4° C   |
|  | Remark:      | 298.4° F   |
|  | Reliability: | score = 2, reliable with restriction; data from Handbook or collection of data.  |
|  | Reference:   | Sax, I.N. and Lewis, R.J., Sr. (eds). <i>Hawley's Condensed Chemical Dictionary</i> , 11 <sup>th</sup> Edition. New York, NY: Van Nostrand Reinhold Company, 1987. 74. |
- (c) Test material: 1-pentyl acetate (CAS 628-63-7)
- Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
- |  |              |  |
|--|--------------|--|
|  | Value:       | 149.2° C   |
|  | Remark:      | 300.6° F   |
|  | Reliability: | score = 2, reliable with restriction; data from Handbook or collection of data.  |
|  | Reference:   | Bisesi, M.S. 1994. Esters. <i>In</i> : Clayton, G.D., and Clayton, F.E. (eds.) <i>Patty's Industrial Hygiene and Toxicology</i> . 4th ed, Vol. II D, New York, NY: John Wiley & Sons Inc., p 2978. |
- (d) Test material: 1-pentyl acetate, commercial grade
- Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
- |  |              |   |
|--|--------------|---|
|  | Value:       | 149° C  |
|  | Remark:      | 300° F  |
|  | Reliability: | score = 2, reliable with restriction; data from Handbook or collection of data.   |
|  | Reference:   | Lide, D.R. (ed). 1995. <i>CRC Handbook of Chemistry and Physics</i> , 75 <sup>th</sup> ed. Boca Raton, FL: CRC Press Inc., 1995-1996. |
- (e) Test material: 1-pentyl acetate (CAS 628-63-7)
- Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
- |  |              |  |
|--|--------------|--|
|  | Value:       | 148° C at 737 mm Hg  |
|  | Remark:      | 298.4 at 737 mm Hg   |
|  | Reliability: | score = 2, reliable with restriction; data from Handbook or collection of data.  |
|  | Reference:   | Verschuere, K. 2001. <i>Handbook of Environmental Data on Organic Chemicals</i> , 4 <sup>th</sup> edition. New York, NY: John Wiley & Sons, Inc. |
- (f) Critical study and preferred value (for minor component only)
- Test Material: 2-methyl-1-butyl acetate (CAS #624-41-9)
- Remark: 2-methyl-1-butyl acetate is the minor component of Primary Amyl Acetate, and is present in the mixture at a concentration of 35%
- |  |              |   |
|--|--------------|---|
|  | Value:       | 140 °C  |
|  | Remark:      | 284 °F  |
|  | Reliability: | Score = 2, valid with restriction; result from internal database.   |
|  | Reference:   | U.S. EPA. 2003. <i>EPI Suite Software</i> , version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and |

Toxics, Washington, DC. Available at:

<http://www.epa.gov/oppt/exposure/docs/episuite.htm>

### 2.3 DENSITY

(a) Critical study and preferred result (for the mixture)

Test material: Primary Amyl Acetate, purity 99.52%; mixture of 60.46% 1-pentyl acetate and 39.06% 2-methyl butyl acetate

Value: 0.87703 g/cm<sup>3</sup>

Temperature: 20° C

Method other: ASTM method D4052: Standard Test Method for Density and Relative Density of Liquids by Digital Density Meter, Annual Book of ASTM Methods Vol. 5.02, 2004.

Year: 2004

Reliability: Score = 1, valid without restriction; meets national standard methods.

Reference: Olson, J., Wilson, L. and Morrison, R. 2006. Physical Properties Measurements on Amyl Series Chemicals: Amyl Alcohol, Amyl Acetate, and Isopentanoic Acid. Report Code SC-AL.SC-2006-000002, dated January 4, 2006.

(b) Critical study and preferred result (for major component only)

Test material: 1-pentyl acetate (CAS 628-63-7)

Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%

Value: 0.879 g/cm<sup>3</sup>

Temperature: 20° C

Method other: no data

Year:

Reliability: score = 2, reliable with restriction; data from Handbook or collection of data.

Reference: Sax, I.N. and Lewis, R.J., Sr. (eds). Hawley's Condensed Chemical Dictionary, 11<sup>th</sup> Edition. New York, NY: Van Nostrand Reinhold Company, 1987. 74.

(c) Test material: 1-pentyl acetate (CAS 628-63-7)

Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%

Value: 0.879 g/cm<sup>3</sup>

Temperature: 20° C

Reliability: score = 2, reliable with restriction; data from Handbook or collection of data.

Reference: Verschueren, K. 2001. Handbook of Environmental Data on Organic Chemicals, 4th edition. New York, NY: John Wiley & Sons, Inc. p.197.

(d) Test material: 1-pentyl acetate (CAS 628-63-7)

Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%

Value: 0.8756 g/cm<sup>3</sup>  
 Reliability: score = 2, reliable with restriction; data from Handbook or collection of data.  
 Reference: Bisesi, M.S. 1994. Esters. *In*: Clayton, G.D., and Clayton, F.E. (eds.) Patty's Industrial Hygiene and Toxicology. 4th ed, Vol. II D, New York, NY: John Wiley & Sons Inc., p 2978.  
 Lide, D.R. (ed). 1995. CRC Handbook of Chemistry and Physics, 76<sup>th</sup> ed. Boca Raton, FL: CRC Press Inc., 1995-1996.

## 2.4 VAPOUR PRESSURE

### (a) Critical study and preferred result (for the mixture)

Test material: Primary Amyl Acetate, purity 99.52%; mixture of 60.46% 1-pentyl acetate and 39.06% 2-methyl butyl acetate  
 Value: 5.73 hPa  
 Temperature: 25° C  
 Remark: 4.298 mm Hg at 25°C  
 Method: ASTM E1719: Standard Test Method for Vapor Pressure of Liquids by Ebulliometry. Annual Book of ASTM Standards, Vol. 14.02.  
 Results: Pressures were measured and controlled with a calibrated Mensor Model PCS 400 manostate to +/- 13 Pa. Temperatures were measured with a calibrated Omega platinum resistance thermometer to 0.01 +/- K. Results are presented below in tabular form

Vapor Pressure Determination for Primary Amyl Alcohol

Pressure in mm Hg	Pressure in KPa	Temperature (°C)
3.066	0.409	20.0
3.307	0.441	21.1
4.298	0.573	25.0

Reliability: score = 1, valid without restriction; meets national standard methods.  
 Reference: Olson, J., Wilson, L. and Morrison, R. 2006. Physical Properties Measurements on Amyl Series Chemicals: Amyl Alcohol, Amyl Acetate, and Isopentanoic Acid. Report Code SC-AL.SC-2006-000002, dated January 4, 2006.

### (b) Critical study and preferred result (for major component only)

Test material: 1-pentyl acetate (CAS 628-63-7))  
 Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%  
 Value: 4.665 hPa  
 Temperature: 25° C  
 Remark: 3.5 mm Hg at 25° F  
 Reliability: score = 2, reliable with restriction; data from Handbook or collection of data.

- Reference: Meylan, W.M. and Howard, P.H. 1997. Handbook of Physical Properties of Organic Chemicals. Boca Raton, FL: Lewis Publishers. p 479.
- Daubert, T.E. and Danner, R.P. 1989. Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Washington, DC: Taylor and Francis. *cited in*: Hazardous Substance Databank (HSDB), updated on 02/02/2000.
- (c) Test material: 1-pentyl acetate (CAS # 628-63-7)  
 Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%  
 Value: 4.665 hPa  
 Temperature: 25° C  
 Remark: 3.50 mm Hg at 25° F  
 Reliability: score = 2, valid with restriction; accepted calculation method  
 Reference: U.S.EPA. 2003. Experimental water solubility and vapor pressure values reported by EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (d) Critical study and preferred result (for minor component only)  
 Test material: 2-methyl-1-butyl acetate (CAS # 624-41-9)  
 Remark: 2-methyl-1-butyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 35%  
 Value: 8.459 hPa  
 Temperature: 25° C  
 Remark: 6.36 mm Hg at 25° F  
 Reliability: Score=2, valid with restriction; accepted calculation method  
 Reference: U.S. EPA. 2003. EPI Suite Software, Version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>

## 2.5 PARTITION COEFFICIENT $\log_{10}K_{ow}$

- (a) Critical study and preferred result (for the mixture)  
 Test material: Primary Amyl Acetate, purity 99.52%; mixture of 60.46% 1-pentyl alcohol and 30.06% 2-methyl-1-butyl alcohol  
 Log P: 2.42  
 Method: equivalent to OECD Test Guideline 107: Measurements were performed in a 0.5 L glass vessel. Water and 1-octanol were added to the vessel through separate 1/16" tubing. The test material was then added to the mixture which was vigorously stirred at 25°C, then allowed to settle for 24 hours. Triplicate samples of each phase were analyzed using a gas chromatograph equipped with a flame ionization detector.  
 Reliability: score = 1, valid without restriction; meets national standard methods

- Reference: Olson, J., Wilson, L. and Morrison, R. 2006. Physical Properties Measurements on Amyl Series Chemicals: Amyl Alcohol, Amyl Acetate, and Isopentanoic Acid. Report Code SC-AL.SC-2006-000002, dated January 4, 2006.
- (b) Critical study and preferred result (for major component only)
- Test material: 1-pentyl acetate (CAS 628-63-7)
- Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
- log K<sub>ow</sub>: 2.30
- Method: measured
- Year: 1994
- Reliability: score = 2, valid with restriction; accepted methodology
- Reference: Abraham, M.H., Chandra, H.S., Whiting, G.S. and Mitchell, R.C. 1994. An analysis of water-octanol and water-alkane partitioning and the delta log P parameter of Seiler. J. Pharm Sci. 83: 1085-1100.
- (c) Test material: 1-pentyl acetate (CAS 628-63-7)
- Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
- log K<sub>ow</sub>: 2.34 at 25° C
- Method: Other, calculated, KOWWIN
- Year:
- GLP: No
- Reliability: Score=2, valid with restrictions; accepted calculation method
- Reference: KOWWIN. Version 1.67. EPI Suite Software, version 3.11, United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (d) Critical study and preferred value (for minor component only)
- Test material: 2-methyl-1-butyl acetate (CAS # 624-41-9)
- Remark: 2-methyl-1-butyl acetate is the minor component of Primary Amyl Acetate, and is present in the mixture at a concentration of 35%
- Log K<sub>ow</sub>: 2.26 at 25° C
- Method: Other, Calculated, KOWWIN
- Year:
- GLP: no
- Reliability: Score=2, valid with restrictions, accepted calculation method
- Reference: KOWWIN. Version 1.67. EPI Suite Software, version 3.11, United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>

## 2.6 WATER SOLUBILITY

- (a) Critical study and preferred result (for the mixture)
- Test material: Primary Amyl Acetate, purity 99.52%; mixture of 60.46% 1-pentyl acetate and 39.06% 2-methyl butyl acetate
- Value: 1700 mg/l at 25° C

## 2. PHYSICAL CHEMICAL PROPERTIES

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- |              |  |
|--------------|--|
| Description: | slightly soluble   |
| Remark:      | reported as 0.17% at 25°C  |
| Method:      | equivalent to OECD Test Guideline 105  |
|              | Solubility in water measurements were performed in a 0.5 L glass vessel maintained at a constant temperature of 25°C. The test material and water were stirred vigorously and then allowed to settle for 24 hours at 25°C. Triplicate samples of each phase were analyzed using a gas chromatograph equipped with a flame ionization detector. |
| Reliability: | score = 1, valid without restriction; meets national standard methods.   |
| Remark:      |  |
| Reference:   | Olson, J., Wilson, L. and Morrison, R. 2006. Physical Properties Measurements on Amyl Series Chemicals: Amyl Alcohol, Amyl Acetate, and Isopentanoic Acid. Report Code SC-AL.SC-2006-000002, dated January 4, 2006.  |
- (b) Critical study and preferred result (for major component only)
- |                |  |
|----------------|--|
| Test material: | 1-pentyl acetate (CAS 628-63-7)  |
| Remark:        | 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%   |
| Value:         | 2000 mg/L at 20 °C   |
| Description:   | slightly soluble   |
| Remark:        | reported as 0.2% (g per 100 g water at 20 °C)  |
| Reliability:   | score = 2, reliable with restriction; data from Handbook or collection of data.  |
| Reference:     | NIOSH (National Institute for Occupational Safety and Health). 1994. NIOSH Pocket Guide to Chemical Hazards. U.S. Department of Human Services, Washington, DC. DHHS (NIOSH) Publication No. 94-116.                                       |
|                | Cosmetic, Toiletry and Fragrance Association (CTFA). 1984. Submission of unpublished data by CTFA. Cosmetic Ingredient Chemical Description: n-Amyl Acetate. Available for review by contacting the Director , Cosmetic Ingredient Review. |
- (c) Test material: 1-pentyl acetate (CAS 628-63-7)
- |              |   |
|--------------|---|
| Remark:      | 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%  |
| Value:       | 1730 mg/l at 25° C  |
| Description: | slightly soluble  |
| Reliability: | score = 2, reliable with restriction; data from Handbook or collection of data.   |
| Reference:   | Yalkowsky, S.H. and Dannenfelser, R.M. 1992. The AQUASOL Databank of Aqueous Solubility, 5 <sup>th</sup> Edition. Tucson, AZ: University of Arizona, College of Pharmacy. cited in: Hazardous Substance Databank (HSDB), updated on 02/02/2000. |
- (d) Test material: 1-pentyl acetate (CAS 628-63-7)
- |         |  |
|---------|--|
| Remark: | 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65% |
| Value:  | 1800 mg/l at 20° C   |



- |              |  |
|--------------|--|
| Description: | slightly soluble   |
| Reliability: | score = 2, reliable with restriction; data from Handbook or collection of data.  |
| Reference:   | Verschueren, K. 2001. Handbook of Environmental Data on Organic Chemicals, 4 <sup>th</sup> edition. New York, NY: John Wiley & Sons, Inc. p.197. |
- (e) Critical study and preferred result (for minor component only)
- |                |  |
|----------------|--|
| Test material: | 2-methyl-1-butyl acetate (CAS #624-41-9)   |
| Remark:        | 2-methyl-1-butyl acetate is the minor component of Primary Amyl Acetate, and is present in the mixture at a concentration of 35%   |
| Value:         | 1070 to 2361.4 mg/l at 25° C   |
| Method:        | Other, calculated, WSKOW v. 1.41, WATERNT v. 1.01  |
| Year:          | 2000   |
| GLP:           | No   |
| Reliability:   | Score=2, valid with restrictions; accepted calculation methods   |
| Reference:     | WSKOW v. 1.41, WATERNT 1.01. U.S. EPA. 2000. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <a href="http://www.epa.gov/oppt/exposure/docs/episuite.htm">http://www.epa.gov/oppt/exposure/docs/episuite.htm</a> |

## 2.7 FLASH POINT (*liquids*)

- (a) Critical study and preferred result (for the mixture)
- |                |   |
|----------------|---|
| Test material: | Primary Amyl Acetate, purity 99.52%; mixture of 60.46% 1-pentyl acetate and 39.06% 2-methyl butyl acetate   |
| Value:         | 37.22° C  |
| Type:          | closed cup  |
| Method:        | ASTM 93, Standard Test Methods for Flash Point by Pensky-Martens Closed Tester, Book of ASTM Standards, Vol.5.01, 2004. Flash point was measured in a manual Fisher/Tag Pensky-Martens apparatus.                   |
| Year:          | 2004  |
| Remark:        | 99° F   |
| Reliability:   | score = 1, reliable without restriction; national standard method   |
| Reference:     | Olson, J., Wilson, L. and Morrison, R. 2006. Physical Properties Measurements on Amyl Series Chemicals: Amyl Alcohol, Amyl Acetate, and Isopentanoic Acid. Report Code SC-AL.SC-2006-000002, dated January 4, 2006. |
- (b)
- |                |   |
|----------------|---|
| Test material: | primary amyl acetate  |
| Value:         | 41° C   |
| Type:          | open cup  |
| Method:        | ASTM D1310  |
| Remark:        | 106° F  |
| Reliability:   | score = 1, reliable without restriction; national standard method |
| Reference:     | The Dow Chemical Company. Material Safety Data Sheet for Primary  |

Amyl Acetate Mixed Isomers. MSDS# 526, effective date 06/12/2001. The Dow Chemical Company, Midland, MI.

- (c) Critical study and preferred value (for major component only)  
Test material: 1-pentyl acetate, commercial grade  
Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%  
Value: 21° C  
Type: closed cup  
Reliability: score = 2, reliable with restriction; data from Handbook or collection of data.  
Reference: NFPA. 2002. National Fire Protection Association. Fire Protection Guide to Hazardous Materials, 13th edition. NFPA. Quincy, MA.
- (d) Test material: 1-pentyl acetate (CAS 628-63-7)  
Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%  
Value: 25° C  
Type: closed cup  
Remark: 77° F  
Reliability: score = 2, reliable with restriction; data from Handbook or collection of data.  
Reference: Sax, I.N. and Lewis, R.J., Sr. (eds). Hawley's Condensed Chemical Dictionary, 11<sup>th</sup> Edition. New York, NY: Van Nostrand Reinhold Company, 1987. 74.  
  
NIOSH. 1994. NIOSH Pocket Guide to Chemical Hazards. DHHS (NIOSH) Publication 94-116.  
  
Clayton, G.D., F.E. Clayton (eds.) Patty's Industrial Hygiene and Toxicology. Volumes 2A, 2B, 2C, 2D, 2E, 2F: Toxicology. 4th ed. New York, NY: John Wiley & Sons Inc., 1993-1994. 2978.

## 2.8 AUTO FLAMMABILITY (*solid/gases*)

- (a) Critical study and preferred result (for major component only)  
Test material: 1-pentyl acetate, commercial grade  
Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%  
Value: 360° C  
Remark: reported as 680° F, autoignition temperature  
Reliability: score = 2, reliable with restriction; from Handbook or collection of data.  
Reference: NFPA. 2002. National Fire Protection Association. Fire Protection Guide to Hazardous Materials, 13th edition. NFPA. Quincy, MA.

**2.9 FLAMMABILITY****(a) Critical study and preferred result (for the mixture)**

Test material: Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl butyl acetate  
Value: Flammable range 1.1 – 7.5 vol % (11,000-75,000 ppm)  
Remark: commercial grade  
Reliability: score = 4, not assignable  
Remark: Data presented by the manufacturer for the mixture of two isomers are consistent with independently published values for its major component, 1-pentyl acetate.  
Reference: The Dow Chemical Company. Material Safety Data Sheet for Primary Amyl Acetate Mixed Isomers. MSDS# 526, effective date 06/12/2001. The Dow Chemical Company, Midland, MI.

**(b)** Test material: 1-pentyl acetate  
Value: Flammable range 1.1 – 7.5 vol % (11,000-75,000 ppm)  
Remark: NFPA lists pentyl acetate as commercial grade  
Reliability: score = 2, reliable with restriction; data from Handbook or collection of data.  
Reference: NFPA. 2002. National Fire Protection Association. Fire Protection Guide to Hazardous Materials. 13th edition. NFPA. Quincy, MA.

**2.10 EXPLOSIVE PROPERTIES**

Test material: Primary Amyl Acetate  
Results: Explosive under influence of flame  
Remarks: Vapors may form explosive mixtures with air; containers may explode when heated.  
Reference: Primary Amyl Acetate (CAS 628-63-7). Hazardous Substance Databank (HSDB), updated on 02/02/2000

**2.11 OXIDIZING PROPERTIES**

Remark: Primary Amyl Acetate is not an oxidizer. Incompatible with strong oxidizing agents. Incompatible with nitric acid, sodium hydroxide, alkali metal hydroxides.  
Reliability: score = 4. Data from manufacturer consistent with data for its major component, 1-pentyl acetate.  
Reference: The Dow Chemical Company. Material Safety Data Sheet for Primary Amyl Acetate Mixed Isomers. MSDS# 526, effective date 06/12/2001. The Dow Chemical Company, Midland, MI.  
  
Remark: Not an oxidizer. Can react with oxidizing materials. Incompatible with nitrates and strong oxidizers.  
  
Reliability: score = 2, reliable with restriction; data from Handbook or collection of data  
  
Reference: Cosmetic, Toiletry and Fragrance Association (CTFA). 1984. Submission of unpublished data by CTFA. Cosmetic ingredient. Chemical description.

Amyl acetate. Available for review: Director, Cosmetic Ingredient Review, 1110 Vermont Ave., N.W. Suite 810, Washington, DC. *cited in*: Elder, R.L. (ed). 1988. Safety Assessment of Cosmetic Ingredients. Final Report on the Safety Assessment of Amyl Acetate and Isoamyl acetate. J. American College of Toxicology 7: 705-719.

## 2.12 ADDITIONAL REMARKS

Remark: Very soluble in ethanol and ether.  
Reliability: score = 2, reliable with restriction; data from Handbook or collection of data  
Reference: Lide, D.R. (ed). 1995. CRC Handbook of Chemistry and Physics, 76<sup>th</sup> ed. Boca Raton, FL: CRC Press Inc. cited in Hazardous Substance Databank (HSDB), updated on 02/02/2000.

Remark: Odor characteristic: sweet, ester, banana, pleasant  
Reliability: score = 2, reliable with restriction; data from Handbook or collection of data  
Reference: Verschuere, K. Data on Organic Chemicals, 2001. Handbook of Environmental 4<sup>th</sup> edition. New York, NY: John Wiley & Sons, Inc. p.197.

## 2.13 ADDITIONAL DATA

Remark: Viscosity at 11° C: 1.58 cPa·sec  
Evaporation rate at 35° C: 50% in 15.5 min, 95% in 40 min  
Reference: HSDB (Hazardous Substance Database), 2002. n-Amyl Acetate, CASRN 628-63-7. HSDB No. 5126. Complete update on 7/22/02.

Remark: Vapour density: 4.5 (air = 1.0)  
Reference: NFPA. 2002. National Fire Protection Association. Fire Protection Guide to Hazardous Materials, 13th edition. NFPA. Quincy, MA.

Clayton, G.D., F.E. Clayton (eds.) Patty's Industrial Hygiene and Toxicology. Volumes 2A, 2B, 2C, 2D, 2E, 2F: Toxicology. 4th ed. New York, NY: John Wiley & Sons Inc., 1993-1994. 2978.

Remark: Lower explosion limit (LEL): 1.1 vol % (11,000 ppm)  
Upper explosion limit (UEL): 7.5 vol % (75,000 ppm)  
Reference: NFPA. 2002. National Fire Protection Association. Fire Protection Guide to Hazardous Materials, 13th edition. NFPA. Quincy, MA.

Remark: 1 ppm is equivalent to 5.32 mg/m<sup>3</sup>  
Reference: Bisesi, M.S. 1994. Esters. *In*: Clayton, G.D., and Clayton, F.E. (eds.) Patty's Industrial Hygiene and Toxicology. 4th ed, Vol. II D, New York, NY: John Wiley & Sons Inc.

### 3.0 ENVIRONMENTAL FATE AND PATHWAYS

#### 3.1 STABILITY

##### 3.1.1 PHOTODEGRADATION

(a) Critical study and preferred value (for major component only)

Atmospheric photo-oxidation

Test substance: 1-pentyl acetate (CAS 628-63-7)

Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%

Type: air

Light source: other

Relative intensity: based on intensity of sunlight

Result:  $t_{1/2} = 35$  hours ( $25^{\circ}$  C), assumed 12 hours daylight,  $1.5E-6$  HO molecules/cm<sup>3</sup>

Degradation product: not measured

Method: other (measured); pulsed laser photolysis laser-induced fluorescence

GLP: no data

Test substance: 1-pentyl acetate (CAS 628-63-7), 99% purity (Aldrich Chemical Co.). Test substance further purified prior to testing.

Method: Pulsed laser photolysis-laser-induced fluorescence apparatus and procedures were used as described by Mellouki et al (1994, 1995). OH radicals were produced by photolysis of H<sub>2</sub>O<sub>2</sub> via laser at 248 nm. The helium carrier gas was UHP-certified (>99.999% pure). 1-Pentyl acetate was pre-mixed with helium to form a 0.3-8% mixture, in a 10 L gas light-tight bulb. The total pressure inside the bulb was approximately 850 Torr. The acetate concentration range was 0.23-2.76 x10<sup>14</sup> molecules/cm and the OH initial concentration was <6 x 10<sup>11</sup> molecules/cm.

The OH precursor (H<sub>2</sub>O<sub>2</sub>), 1-pentyl acetate, and helium were introduced into the reaction cell via Teflon tubing. As the 1-pentyl acetate was found to be “sticky” at room temperature, the bulb and Teflon lines were heated to about 50 degree C. The gasses flowed through the reaction cell at a linear velocity range of 5-20 cm/sec orthogonal to the photolysis and probe laser beams. Flow rates and cell pressures were measured. Fluorescence was collect orthogonal to both beams and focused through lenses and a band-pass filter (309.4 nm), onto a photomultiplier (PM) tube. The output signal from the PM tube was integrated for a set period, digitised, and computer averaged and analysed. The signals from 100 probe laser events were averaged to obtain one data point. The flow of the reactants was sufficiently slow to allow each photolysis/probe sequence to occur with a fresh gas mixture, therefore reaction products did not build up within the reaction cell.

Results:	<p>The kinetic reaction of 1-pentyl acetate and OH radicals was studied under first-order conditions. Systematic errors were minimized as much as possible. Secondary reactions of OH with initial reaction products were reduced by the large (acetate:OH) ratio used. Rate constants were demonstrated to be independent of system pressure changes or variations in the flow rates through the reaction cell. The influence of impurities was negligible, given the high purity of the test substance and carrier gas used.</p> <p>The Arrhenius equation derived from the obtained kinetic data was: <math>K = (2.75 \pm 0.46) \times 10^{-12} \exp[(302 \pm 102)/T]</math> (in units of <math>\text{cm}^3/\text{molecule} \cdot \text{second}</math>)</p> <p>The rate constant for the reaction of 1-pentyl acetate with OH radicals was determined over a temperature range of 243-372 K. The room temperature rate constant of <math>7.34 (\pm 0.91) \times 10^{-12} \text{ cm}^3/\text{molecule} \cdot \text{second}</math> at 298 K was very comparable to the value of <math>7.53 (\pm 0.48) \times 10^{-12} \text{ cm}^3/\text{molecule} \cdot \text{second}</math> at 297 K, that was reported by Williams et al in 1993. (Williams, C.C., O'Rji, L.N. and Stone, D.A. 1993. Int. J. Chem Kinet. 25: 539-548).</p>
Reliability:	score = 2, valid with restriction; data from Handbook or collection of data; accepted calculation method
Reference:	El Boudali, S., Le Calve, S., Le Bras, G. and Mellouki, A. 1996. Kinetic studies of OH reactions with a series of acetates. J. Phys. Chem. 100: 12364-12368.
(b) Atmospheric photo-oxidation	
Test substance:	1-pentyl acetate (CAS 628-63-7)
Remark:	1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
Type:	other
Light source:	
Relative intensity:	based on intensity of sunlight
Degradation products:	not measured
Method:	other (calculated) AOPWIN v.1.91
GLP:	no
Remark:	Vapor-phase 1-pentyl is expected to be degraded in the atmosphere by reaction with photochemically-produced hydroxyl (OH) radicals. The 2nd order rate constant was calculated as $7.55 \times 10^{-12} \text{ cm}^3/(\text{molecule} \cdot \text{sec})$ at 25 deg. C. Based on $1.5 \times 10^6 \text{ OH molecules/cm}^3$ and assuming 12 hours of sunlight per day, the estimated troposphere half-life is 34.0 hours or 1.42 days.
Reliability:	score = 2, valid with restriction; data from Handbook or collection of data; accepted calculation method, this value self selected by the Level III distribution model by default.
Reference:	Kwok, E.S.C. and R. Atkinson. 1995. Estimation of hydroxyl radical reaction rate constants for gas-phase organic compounds using a

structure-reactivity relationship: An update. Atmospheric Environment. 29(14):1685-1695.

AOPWIN, v. 1.91. U.S. EPA. 2003. EPI Suite Software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>

(c) Atmospheric photo-oxidation

Test substance: 1-pentyl acetate (CAS 628-63-7)

Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%

Type: other

Light source:

Relative

intensity: based on intensity of sunlight

Degradation

products: not measured

Method: other (calculated) AOPWIN v.1.91

GLP: no

Remark: The atmospheric photo-oxidation potential of 1-pentyl acetate was estimated using the submodel AOPWIN which relies on structural features of the test substance. The model calculates a second order half-life with units of cm<sup>3</sup>/molecules-cm. Vapor phase 1-pentyl acetate is expected to degrade in the atmosphere by reaction with photochemically produced hydroxyl (OH) radicals. The 2<sup>nd</sup> order rate constant was calculated as 6.0225E-12 cm<sup>3</sup>/(molecule\*sec) at 25° C. Based on 1.5 x 10<sup>6</sup> OH molecules/cm<sup>3</sup> and assuming 12 hours of sunlight per day, the estimated half-life was 1.776 days or 42.624 hours.

Reliability: score = 2, reliable with restriction; accepted calculation method

Reference: AOPWIN, v. 1.91. U.S. EPA. 2003. EPI Suite Software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>

(d) Atmospheric photooxidation

Test substance: 1-pentyl acetate (CAS 628-63-7)

Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%

Type: air

Degradation

products: not measured

Method: other (estimation)

GLP: no

Test substance: 1-pentyl acetate (CAS 628-63-7)

Remark: Abiotic degradation: The rate constant for the vapour phase reaction of n-pentyl acetate with photochemically produced hydroxy radicals

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	has been estimated as $6.0 \times 10^{-12}$ cm <sup>3</sup> /molecule*sec at 25° C. This corresponds to an atmospheric half-life of 2.83 days at an atmospheric concentration of $5 \times 10^5$ hydroxy radicals/cm <sup>3</sup> .
Reliability:	score = 2, reliable with restriction; accepted calculation method
Reference:	Meylan, W.M. and Howard, P.H. 1993. Computer estimation of the atmospheric gas-phase reaction rate of organic compounds with hydroxy radicals and ozone. Chemosphere 26: 2293-2299. cited in: Hazardous Substance Databank (HSDB), updated on 02/02/2000.
	Howard, P.H. 1990. Handbook of Environmental Fate and Exposure Data for Organic Chemicals. Volume II Solvents. Chelsea, MI: Lewis Publishers, Inc.
(e)	Critical study and preferred value (for minor component only)
	Atmospheric photo-oxidation
Test material:	2-methyl-1-butyl acetate (CAS 624-41-9)
Remark:	2-methyl-1-butyl acetate is the minor component of Primary Amyl Acetate, and is present in the mixture at a concentration of 35%
Type:	other
Light source:	
Relative intensity:	based on intensity of sunlight
Degradation products:	not measured
Method:	other (calculated) AOPWIN v.1.91
GLP:	no
Remark:	The atmospheric photo-oxidation potential of 2-methyl butyl acetate was estimated using the submodel AOPWIN which relies on structural features of the test substance. The model calculates a second order half-life with units of cm <sup>3</sup> /molecules-cm. Vapor phase 2-methyl butyl acetate is expected to degrade in the atmosphere by reaction with photochemically produced hydroxyl (OH) radicals. The 2 <sup>nd</sup> order rate constant was calculated as $6.2987\text{E-}12$ cm <sup>3</sup> /(molecule*sec) at 25° C. Based on $1.5 \times 10^6$ OH molecules/cm <sup>3</sup> and assuming 12 hours of sunlight per day, the estimated half-life was 1.698 days or 40.752 hours.
Reliability:	score = 2, reliable with restriction; accepted calculation method
Reference:	AOPWIN, v1.91 U.S.EPA. 2003. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <a href="http://www.epa.gov/oppt/exposure/docs/episuite.htm">http://www.epa.gov/oppt/exposure/docs/episuite.htm</a>

## 3.1.2 STABILITY IN WATER

(a)	Critical study and preferred Value (for the mixture)
Test substance:	Primary Amyl Acetate, purity 99.6%
Type:	abiotic hydrolysis
Method:	OECD Guideline 111: Hydrolysis as a Function of pH, 1981. EEC Commission Directive 87/302/EEC
Year:	1981



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GLP:	yes
t1/2 pH4	84.8 days
t1/2 pH7	138 days
t1/2 pH9	21.9 days
Remark:	<p>The hydrolysis kinetics of Primary Amyl Acetate was evaluated at 50, 60, and 70 °C in 0.05 M buffered solutions at pH 4, 7, and 9. The buffered solutions were prepared with potassium biphthalate, potassium phosphate, and sodium borate, respectively. Precautions were taken to minimize biodegradation, oxidation, and photodegradation reactions.</p> <p>A Tier I probe was conducted at 50 °C at pH 4, 7, and 9. Each buffered solution was dosed with 102 mg/L Primary Amyl Acetate. After 5 days, 76.5, 85.0, and 0.0 mg/L of the test material remained at pH 4, 7, and 9, respectively. This translated into half-lives of 2900, 36.1, and 0.8 days, at pH 4, 7, and 9, respectively. These measured half lives at 50 °C can be extrapolated at to half lives between 24 hours and 1 year at 25 °C. More extensive kinetic evaluations were required to comply with test guidelines.</p> <p>Tier II evaluations were conducted in pH 4, 7, and 9 buffers at 50, 60, and 70 °C. Hydrolysis rates for Primary Amyl Acetate increased with temperature and followed pseudo-first order kinetics. Buffered solutions were dosed with approximately 100 mg/L Primary Amyl Acetate. Half lives at 50, 60, and 70 °C were determined to be 30.1, 19.3, and 14.4 days at pH 4; 18.1, 11.1, and 4.6 days at pH 7; and 0.8, 0.3, and 0.1 days at pH 9. Ursing the Arrhenius relationship (logarithm of pseudo-first order rate constant vs. reciprocal of temperature in °K), the predicted hydrolysis half-life at 25 °C in pH 4, 7, and 9 buffered solutions are 84.8, 138, and 21.9 days, respectively.</p>
Reliability:	score = 1, reliable without restriction, GLP guideline study.
Reference:	Davis, J.W. and Marty, G.T. 2004a. Primary Amyl Acetate: Evaluation of Hydrolysis as a Function of pH Using OECD Guideline 111 (unpublished study). Toxicology & Environmental Research and Consulting, Study ID 031069. The Dow Chemical Company, Midland, MI.
(b) Critical study and preferred Value (for minor component only)	
Test substance:	1-pentyl acetate (CAS 628-63-7)
Remark:	1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
Type	Abiotic (hydrolysis)
t1/2 pH4	
t1/2 pH7	2.142 yr at 25° C
t1/2 pH8	78.232 days at 25° C
t1/2 pH9	
Method:	other (calculated) HYDROWIN v.1.67
Year:	
GLP:	no
Test substance:	1-pentyl acetate (CAS 628-63-7)
Remarks:	The rate constant for pH >8 at 25 degree C = $1.025 \times 10^{-1}$ L/molecule*second.

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Reliability:	score = 2, reliable with restriction; accepted calculation method
Reference:	HYDROWIN. Version 1.67. Aqueous hydrolysis rate estimate from chemical structure. EPI Suite Software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <a href="http://www.epa.gov/oppt/exposure/docs/episuite.htm">http://www.epa.gov/oppt/exposure/docs/episuite.htm</a>
(c) Test substance:	1-pentyl acetate (CAS 628-63-7)
Remark:	1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
Type	Abiotic (hydrolysis)
t1/2 pH4	
t1/2 pH7	
t1/2 pH8	
t1/2 pH9	13.5 days
Method:	other (estimated)
Year:	1990
GLP:	no
Test substance:	1-pentyl acetate (CAS 628-63-7)
Remarks:	The hydrolysis rate of 1-pentyl acetate at pH > 8 is estimated to be $5.93 \times 10^{-2}$ L/mol-sec at 25 degree C., resulting in a half-life at pH 9, of 13.5 days. Because simple esters are resistant to hydrolysis, hydrolysis is not expected to be a significant degradation process under environmental conditions.
Reliability:	score = 2, reliable with restriction; accepted calculation method, however, the preferred value relies on an updated SAR, as compared to this calculated value
Reference:	Howard, P.H. 1990. Handbook of Environmental Fate and Exposure Data for Organic Chemicals. Volume II Solvents. Chelsea, MI: Lewis Publishers, Inc.
(d) Critical study and preferred Value (for minor component only)	
Test material:	2-methyl-1-butyl-Acetate (CAS 624-41-9)
Remark:	2-methyl-1-butyl acetate is the minor component of Primary Amyl Acetate, and is present in the mixture at a concentration of 35%
Type	Abiotic (hydrolysis)
t1/2 pH4	
t1/2 pH7	3.334 yr at 25 degree C
t1/2 pH8	121.728 days at 25 degree C
t1/2 pH9	
Method:	other (calculated) HYDROWIN v.1.67
Year:	
GLP:	no
Remarks:	The rate constant for pH >8 at 25 degree C = $6.587 \times 10^{-2}$ L/molecule*second.
Reliability:	score = 2, reliable with restriction; accepted calculation method
Reference:	HYDROWIN. Version 1.67. Aqueous hydrolysis rate estimate from chemical structure. U.S.EPA. 2003. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution

Prevention and Toxics, Washington, DC. Available at:  
<http://www.epa.gov/oppt/exposure/docs/episuite.htm>

### 3.1.3 STABILITY IN SOIL

No data available

## 3.2 MONITORING DATA (ENVIRONMENT)

### (a) Type of Measurement

Test substance: 1-pentyl acetate (CAS 628-63-7)

Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%

Media: water

Remark: In a survey of drinking water in the United Kingdom, 1-pentyl acetate was detected in drinking water in one of 14 plants tested; the source of water for this plant was groundwater.

Reference: Fielding, M. et al. 1981. Organic Micropollutants in Drinking Water. TR-159 Medmenham, English Water Research Centre pp. 49. *Cited in:* Howard, P.H. 1990. Handbook of Environmental Fate and Exposure Data for Organic Chemicals. Volume II Solvents. Chelsea, MI: Lewis Publishers, Inc.

### (b) Type of Measurement

Test substance: 1-pentyl acetate (CAS 628-63-7)

Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%

Media: effluent

Remark: 1-pentyl acetate has been detected in effluent originating from the explosives industry (26 ppm) and from the porcelain/enamelling industry (31 ppm).

Reference: Shackelford, W.M. et al. 1983. Anal Chem Acta 146: 15-27. *Cited in:* Howard, P.H. 1990. Handbook of Environmental Fate and Exposure Data for Organic Chemicals. Volume II Solvents. Chelsea, MI: Lewis Publishers, Inc.

## 3.3 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION PATHWAYS

### 3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

#### (a) Critical study and preferred result (for the mixture)

Test substance: Primary Amyl Acetate; mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate.

Type: volatility

Method: calculation using EPIWIN (v.3.12) and default model parameters [water depth 1 m (for both lake and river), wind speed 5 m/sec (river)]

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		and 0.5 m/sec (lake), and current 1 m/sec (river and 0.05 m/sec (lake)].
Year:		2000
GLP:		no
Remark:		Volatilization from surface waters: half-life from model river = 3.443 hours half-life from model lake = 5.551 days Default model parameters were used, as were molecular weight 130.19 g/mol, water solubility 1700 mg/L, vapor pressure 4.298 mm Hg at 25°C, and Henry's law constant $2.932 \times 10^{-4}$ atm-m <sup>3</sup> /mol at 25°C.
Reliability:		score = 2, valid with restriction; accepted calculation method
Reference:		EPIWIN (Estimated Program Interface for Windows) Version 3.12. U.S. Environmental Protection Agency, 2000. Available at <a href="http://www.epa.gov/oppt/exposure/docs/episuite.htm">http://www.epa.gov/oppt/exposure/docs/episuite.htm</a>
(b)	Test substance:	1-pentyl acetate (CAS 628-63-7)
	Remark:	1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
	Type:	volatility Calculation based on Henry's law constant of $3.00 \times 10^{-4}$ atm m <sup>3</sup> /mol at 25° C. Estimated half-life = 3.391 hr (model river); 5.528 days (model lake). Model inputs: vapor pressure of 3.5 mm Hg (25° C) and a Henry's Law constant of $3.00 \times 10^{-4}$ atm-m <sup>3</sup> /mol (25° C). Test substance is likely to volatilize fairly easily from fast moving rivers, but only moderately from quiescent lakes and other surface waters.
	Reliability:	Score = 2, reliable with restrictions, accepted calculation method
	Reference:	U.S. EPA. 2003. EPI Suite Software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <a href="http://www.epa.gov/oppt/exposure/docs/episuite.htm">http://www.epa.gov/oppt/exposure/docs/episuite.htm</a>
(c)	Test substance:	1-pentyl acetate (CAS 628-63-7)
	Remark:	1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
	Type:	volatility
	Media:	water - air
	Method other:	estimate
	Year:	1990
	Remark:	A volatilisation half-life of 6 hours has been estimated for a river meter deep flowing 1 m/sec with a wind velocity of 3 m/sec A volatilisation half-life of 127 hours has been estimated for a river one meter deep flowing 0.05 m/s with a wind velocity of 0.5 m/sec.
	Reliability:	score = 2, valid with restriction; accepted calculation method; data from Handbook or collection of data.
	Reference:	Lyman, W.J. et al. 1990. Handbook of Chemical Property Estimation Methods. Environmental behaviour of organic compounds. Washington, DC: American Chemical Society.

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- (d) Test substance: 1-pentyl acetate (CAS 628-63-7)  
 Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%  
 Type: volatilisation  
 Method: other (estimated)  
 Year:  
 GLP: no  
 Test substance: 1-pentyl acetate (CAS 628-63-7)  
 Remark: Using the Henry's law constant of  $3.91 \times 10^{-4}$  atm-m<sup>3</sup>/mole at 25 degree C, the estimated volatilisation half-life of 1-pentyl acetate in a model river 1 m deep with a current of 1 m/sec and a 3 m/sec wind speed is 5.9 hours.  
 Reliability: score = 2, reliable with restriction; accepted calculation method  
 Reference: Howard, P.H. 1990. Handbook of Environmental Fate and Exposure Data for Organic Chemicals. Volume II Solvents. Chelsea, MI: Lewis Publishers, Inc.  
 Lyman, W.J. et al. 1982. Handbook of Chemical Property Estimation Methods. New York: McGraw Hill.
- (e) Test substance: 2-methyl-1-butyl acetate (CAS 624-41-9)  
 Remark: 2-methyl-1-butyl acetate is the minor component of Primary Amyl Acetate, and is present in the mixture at a concentration of 35%  
 Type: volatility  
 Calculation based on Henry's law constant of  $5.35 \times 10^{-4}$  atm m<sup>3</sup>/mol at 25 deg. C (calculated by the model), and molecular weight 130.19 g/mol. Estimated half-life = 2.413 hr (model river); 5.083 days (model lake). Test substance is likely to volatilize fairly easily from fast moving rivers, but only moderately from quiescent lakes and other surface waters.  
 Reliability: score=2, reliable with restrictions, accepted calculation method  
 Reference: U.S.EPA. 2003. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at:  
<http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (f) Test substance: 1-pentyl acetate (CAS 628-63-7)  
 Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%  
 Type: volatility  
 Media: soil - air  
 Method other: estimate  
 Year: 1989  
 Remark: In ambient atmosphere, 1-pentyl acetate is expected to volatilise from dry soil surfaces based on a vapour pressure of 3.5 mm Hg at 25° C.  
 Reliability: score = 2, reliable with restriction; data from Handbook or collection of data

Reference:	Daubert, T.E. and Danner, R.P. 1989. Physical and Thermodynamic Properties of Pure Chemicals: Data Compilation. New York, NY: Hemisphere Publishing Corp.
	Howard, P.H. (ed.). 1990. Handbook of Environmental Fate and Exposure Data for Organic Chemicals. Volume II Solvents. Lewis Publishers. Chelsea, Michigan.
(g) Test substance:	1-pentyl acetate (CAS 628-63-7)
Remark:	1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
Type:	
Dissipation time:	
Method:	
Test substance:	1-pentyl acetate
Remarks:	Volatilisation from dry soil surfaces is expected based on the vapour pressure of this compound, and volatilisation from moist surfaces is expected based on its Henry's Law constant. Based upon an estimated Koc value of 73 determined from a calculated log Kow of 2.3, 1-pentyl acetate is expected to have low to moderate mobility in soil. This compound is expected to biodegrade in the soil environment, based upon results from screening biodegradation studies.
Reliability:	score = 2, reliable with restriction; data from Handbook or collection of data.
Reference:	Howard, P.H. 1990. Handbook of Environmental Fate and Exposure Data for Organic Chemicals. Volume II Solvents. Chelsea, MI: Lewis Publishers, Inc. Lyman, W.J. et al. 1982. Handbook of Chemical Property Estimation Methods. New York: McGraw Hill.

### 3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

(a) Critical study and preferred value (for the mixture)	
Test substance:	Primary Amyl Acetate; mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-pentyl acetate
Media:	other: air, water, soil, and sediment
Method:	calculation, fugacity model level III
Year:	2000
Remark:	Air: half-life = 34.0 hr, emissions = 1000 kg/hr Water: half-life = 208 hr, emissions = 1000 kg/hr Soil: half-life = 416 hr, emissions = 1000 kg/hr Sediment: half-life = 1870 hr, emissions = 0 kg/hr
Persistence Time:	230 hr
Physical properties used for the distribution modelling at 25° C were vapor pressure of 4.298 mm Hg, melting point -94 deg. C, and boiling point 148.4° C, water solubility 1700 mg/L, log Kow 2.42, and Henry's Law Constant $2.932 \times 10^{-4}$ atm-m <sup>3</sup> /mol. Half-lives were calculated by the model. All other model parameters were default values or were calculated by EPISuite.	
Air (Level III):	6.84%
Water (Level III):	26.7%

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- |                   |   |
|-------------------|---|
| Soil (Level III): | 66.3%   |
| Sediment:         | 0.141%  |
| Reliability:      | Score=2, reliable with restrictions, accepted calculation method  |
| Reference:        | U.S.EPA. 2000. EPI Suite software, version 3.12. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at:<br><a href="http://www.epa.gov/oppt/exposure/docs/episuite.htm">http://www.epa.gov/oppt/exposure/docs/episuite.htm</a> |
- (b) Critical study and preferred value (for major component only)
- |                   |  |
|-------------------|--|
| Test substance:   | 1-pentyl acetate (CAS 628-63-7)  |
| Remark:           | 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%   |
| Media:            | other: air, water, soil, and sediment  |
| Method:           | calculation, fugacity model level III  |
| Year:             | 2003   |
| Remark:           | Air: half-life = 34.0 hr, emissions = 1000 kg/hr<br>Water: half-life = 208 hr, emissions = 1000 kg/hr<br>Soil: half-life = 208 hr, emissions = 1000 kg/hr<br>Sediment: half-life = 832 hr, emissions = 0 kg/hr |
| Persistence Time: | 160 hr   |
- Physical properties used for the distribution modelling at 25° C were vapor pressure of 3.5 mm Hg, melting point -70.8 deg. C, and boiling point 148.4° C, water solubility 2000 mg.L (20° C), log Kow 2.34, and Henry's Law Constant 3.00E-4 atm-m<sup>3</sup>/mol. Half-lives were calculated by the model. All other model parameters were default values or were calculated by EPISuite.
- |                    |   |
|--------------------|---|
| Air (Level III):   | 9.38%   |
| Water (Level III): | 37.7%   |
| Soil (Level III):  | 52.7%   |
| Sediment:          | 0.129%  |
| Reliability:       | Score=2, reliable with restrictions, accepted calculation method  |
| Reference:         | U.S.EPA. 2000. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at:<br><a href="http://www.epa.gov/oppt/exposure/docs/episuite.htm">http://www.epa.gov/oppt/exposure/docs/episuite.htm</a> |
- (b) Preferred value (for minor component only)
- |                   |   |
|-------------------|---|
| Test material:    | 2-methyl-1-butyl acetate (CAS 624-41-9)   |
| Remark:           | 2-methyl-1-butyl acetate is the minor component of Primary Amyl Acetate, and is present in the mixture at a concentration of 35%  |
| Media:            | other: air, water, soil, and sediment   |
| Method:           | calculation, fugacity model level III   |
| Year:             | 2003  |
| Remark:           | Air: half-life = 40.8 hr, emissions = 1000 kg/hr<br>Water: half-life = 360 hr, emissions = 1000 kg/hr<br>Soil: half-life = 360 hr, emissions = 1000 kg/hr<br>Sediment: half-life = 1440 hr, emissions = 0 kg/hr |
| Persistence Time: | 204 hr  |

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Physical properties used for the distribution modelling at 25 deg. C were melting point - 56°C, boiling point 140°C, vapor pressure of 6.36 mm Hg, log Kow of 2.26, water solubility 1070 mg/L, and Henry's Law constant of 5.35E-4 atm-m<sup>3</sup>/mol. Soil Koc and half-lives were calculated by the model. All other model parameters were default values or were calculated by EPISuite.

Air (Level III): 10.4%

Water (Level III): 36.9%

Soil (Level III): 52.6%

Sediment: 0.145%

Reliability: (2) reliable with restrictions, accepted calculation method

Reference: U.S.EPA. 2000. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>

- (c) Test substance: 1-pentyl acetate (CAS 628-63-7)
- Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
- Media other: air - suspended atmospheric particles
- Method other: estimate
- Year:
- Remark: According to a model of gas/particle partitioning of semivolatile organic materials in the atmosphere, 1-pentyl acetate is expected to exist solely as a vapour in the ambient atmosphere, based on a vapour pressure of 3.5 mm Hg at 25° C.
- Reliability: score = 2, reliable with restriction; accepted calculation method; data from Handbook or collection of data
- Reference: Daubert, T.E. and Danner, R.P. 1989. Physical and Thermodynamic Properties of Pure Chemicals: Data Compilation. New York, NY: Hemisphere Publishing Corporation.

## 3.3.3 OTHER DISTRIBUTION

- (a) Critical study and preferred value (for major component only)
- Test substance: 1-pentyl acetate (CAS 628-63-7)
- Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
- Type: Abiotic adsorption
- Method: other (estimated)
- Year:
- GLP: no
- Remark: An estimated Koc value of 73 has been determined using water solubility (1700 mg/L, chosen by Howard, 1990) and recommended regression equations. Based on this value, 1-pentyl acetate is not expected to adsorb to suspended solids and sediment in water.
- Reliability: score = 2, reliable with restriction; accepted calculation method



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- Reference: Howard, P.H. 1990. Handbook of Environmental Fate and Exposure Data for Organic Chemicals. Volume II Solvents. Chelsea, MI: Lewis Publishers, Inc.  
Lyman, W.J. et al. 1982. Handbook of Chemical Property Estimation Methods. New York: McGraw Hill.
- (b) Test substance: 1-pentyl acetate (CAS 628-63-7)  
Remark: 1-pentyl acetate is the major component of Primary AmylAcetate, and is present in the mixture at a concentration of 65%  
other: Koc = 38.47 (calculated)  
Remark: Calculated from molecular structure  
Reliability: score = 2, reliable with restrictions, accepted calculation method  
Reference: PCKOCWIN. Version 1.66  
U.S.EPA. 2000. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- c) Critical study and preferred value (for minor component only)  
Test material: 2-methyl-1-butyl acetate (CAS 624-41-9)  
Remark: 2-methyl-1-butyl acetate is the minor component of Primary Amyl Acetate, and is present in the mixture at a concentration of 35%  
other: Koc = 33.78 (calculated)  
Remark: Calculated from molecular structure  
Reliability: score = 2, reliable with restrictions, accepted calculation method  
Reference: PCKOCWIN. Version 1.66 U.S.EPA. 2000. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>

**3.4 IDENTIFICATION OF MAIN MODE OF DEGRADABILITY IN ACTUAL USE****3.5 BIODEGRADATION**

- (a) Critical study and preferred value (for the mixture)  
Test substance: Primary Amyl Acetate, mixed isomers, purity 99.6% (mixture of 65% 1-pentyl acetate, CAS 628-63-7, and 35% 2-methyl-1-butyl acetate, CAS 624-41-9).  
Type: aerobic  
Inoculum: domestic wastewater secondary effluent  
Concentration: 2.8 mg/l  
Contact time: 28 days  
Degradation: 57.1% after 28 days  
Results: biodegrades, but does not meet criteria for “readily biodegradable”  
Kinetic: 3 day =  $31.1 \pm 0.2\%$   
5 day =  $36.7 \pm 0.6\%$   
10 day =  $49.4 \pm 3.5\%$   
21 day =  $52.8 \pm 0.6\%$   
28 day =  $57.1 \pm 6.9\%$

Control: sodium benzoate, 4.02 mg/l  
 Method: OECD Guidelines for Testing of Chemicals. 1992.  
 Guideline No. 301D: Ready Biodegradability, Closed Bottle Test.

GLP: yes  
 Year: 2004  
 Method: Mineral medium specified by OECD Guideline 301D was prepared in deionised Milli-Q<sup>®</sup> water (ingredients and preparation methods described in detail in report). The microbial inoculum consisted of secondary effluent, collected from City of Midland municipal domestic wastewater treatment plant. Effluent was collected one day prior to test initiation, and filtered through Whatman 114V filter paper prior to use. Mineral medium was inoculated with the filtered effluent at a concentration of 4 ml/L; a total of 16 liters of inoculated mineral medium was prepared. The inoculated mineral medium was aerated for a minimum of 30 minutes, and allowed to stand at test temperature for at least 20 hours prior test initiation. The pH of the inoculated mineral medium was 7.4 and did not require adjustment. Dissolved oxygen concentration in the equilibrated, inoculated mineral medium was 8.9 mg O<sub>2</sub>/L prior to test initiation.

Inoculum blanks were prepared by volumetrically removing 6 liters of inoculated mineral medium and dispensing into 300 ml glass BOD bottles. A procedure control suspension containing 4.02 mg sodium benzoate per liter of inoculated mineral medium was prepared and dispensed into 300 ml glass BOD bottles. Test suspensions were prepared by adding 21 ml of a 1065 mg/L stock solution of Primary Amyl Acetate (mixed isomers) in Milli-Q<sup>®</sup> water to 8 liters of inoculated mineral medium for a final concentration of 2.80 mg/L. Test suspensions were dispensed into 300 ml glass BOD bottles. Toxicity control suspensions containing 4.02 mg/L sodium benzoate and 2.80 mg/L Primary Amyl Acetate (mixed isomers) were also prepared and dispensed into 300 ml glass BOD bottles (see table below for experimental matrix). BOD bottles were filled to capacity, and sealed with ground glass stoppers and plastic covers to prevent entrapment of air bubbles and evaporation of the aqueous media.

#### Matrix for Evaluation of Biodegradability of Primary Amyl Acetate

Description of Mixture	Contents	Sampling Interval (days) <sup>1</sup>	Number of Replicates
Inoculum blanks	Inoculated mineral medium	0,3,5,7,10,14,17,21,28	18
Test suspensions	Inoculated mineral medium + 2.8 mg/L primary amyl acetate (mixed isomers)	0,3,5,7,10,14,17,21,28	18
Procedure controls	Inoculated mineral medium + 4 mg/L sodium benzoate	0, 7, 14	6
Toxicity controls	Inoculated mineral medium + 4 mg/L sodium benzoate + 2.8 mg/L primary amyl acetate (mixed isomers)	0, 7, 14	6

1: two 300-ml BOD bottles from each group sampled at each interval

Inoculum blanks were used for duplicate measurements for each sampling interval (day 0, 3, 5, 7, 10, 14, 17, 21, 28). Blanks were used to determine mean values for oxygen consumption due to endogenous respiration of the inoculum. A total of 18 test suspensions were prepared containing 2.80 mg/L of the test material to allow duplicate measurements at each sampling interval. The concentration of test material at test initiation was not verified by chemical analysis, and the concentration remaining at the end of each sampling interval was not determined.

Procedure control mixtures which contained 4.02 mg/L sodium benzoate were sampled in duplicate on days 0, 7, and 14. Similarly, six replicate toxicity control bottles containing the test material and sodium benzoate were prepared and sampled on days 0, 7, and 14.

Reaction mixtures were statically incubated in a walk-in incubator which was maintained in complete darkness except when dissolved oxygen measurements were taken. Air temperature was automatically recorded at 3-hour intervals during the test; the average air temperature was  $20.1 \pm 0.1$  °C. Dissolved oxygen measurements were performed using an Orion model 97-08 dissolved oxygen electrode connected to an Orion model 920A+ pH meter. The meter and electrode were calibrated prior to performing oxygen measurements at each sampling interval. Dissolved oxygen measurements were performed in duplicate for inoculum blanks and test suspensions on days 0, 3, 5, 7, 10, 14, 17, 21, and 28. Dissolved oxygen measurements were performed in duplicate for procedure and toxicity controls on days 0, 7, and 14.

Calculations: The biological oxygen demand (BOD) was determined for each reaction mixture at each sampling interval using the following formula:

$$\text{BOD} = \frac{\text{mg O}_2 \text{ uptake by test substance} - \text{mean mg O}_2 \text{ uptake by blanks}}{\text{mg test substance in vessel}}$$

The percent degradation (%DO<sub>2</sub>) at each sample interval was determined by dividing the BOD by the theoretical oxygen demand (ThOD) for each reaction mixture:

$$\% \text{DO}_2 = \frac{\text{BOD}}{\text{ThOD}} \times 100$$

Where ThOD is the theoretical oxygen demand required for complete mineralization of the test material to carbon dioxide and water.

Statistical methods: descriptive statistics (mean, standard deviation) were used where applicable.

Results: Complete dissolution of the test material was confirmed by dissolved

organic carbon analysis of the test stock suspension. Of the calculated 688 mg carbon/L available, 91% was recovered. Incubation temperatures ranged from 18.6 to 19.7 °C, and averaged  $19.2 \pm 0.3$  °C. Onset of primary amyl acetate biodegradation was rapid and approximately 50% biodegradation was achieved by day 10. The maximum biodegradation achieved was 57.1% on Day 28 of the test. The maximum difference between replicates was 6.9% which occurred on Day 28.

Biodegradation in the procedure controls was  $81.6 \pm 0.7\%$  on day 14. Biodegradation in toxicity controls was 62.6% on day 14, indicating that the test material was not inhibitory on the inoculum.

#### Biodegradation of Primary Amyl Acetate (mixed isomers) at Selected Sampling Intervals

Sampling Interval (days)	% Biodegradation (mean $\pm$ SD, n=2)		
	Test Material	Procedure Control	Toxicity Control
0	0	0	0
3	$31.1 \pm 0.2$		
5	$36.7 \pm 0.6$		
7	$45.5 \pm 0.8$	$76.3 \pm 0.8$	$62.9 \pm 0.1$

#### Biodegradation of Primary Amyl Acetate (mixed isomers) at Selected Sampling Intervals

Sampling Interval (days)	% Biodegradation (mean $\pm$ SD, n=2)		
	Test Material	Procedure Control	Toxicity Control
10	$49.4 \pm 3.5$		
14	$51.4 \pm 1.2$	$81.6 \pm 0.7$	$62.6 \pm 0.1$
17	$51.9 \pm 0.6$		
21	$52.8 \pm 0.6$		
28	$57.1 \pm 6.9$		

#### Remark:

OECD 301D criterion for “readily biodegradable” classification requires reaching 60% biodegradation within a 10-day window, following onset of biodegradation, within a test interval not to exceed 28 days. Maximum biodegradation achieved was 57% after 21 days. Based on these results, primary amyl acetate (mixed isomers) cannot be considered readily biodegradable according to OECD criteria.

OECD 301D guidelines specify that maximum oxygen consumption in inoculum blanks must not exceed 1.5 mg/L over 28 days, and dissolved oxygen levels in test suspensions must not fall below 0.5 mg/L. In this test, maximum oxygen consumption in inoculum blanks after 28 days was only 0.83 mg/L, while the minimum dissolved oxygen concentration observed in the test suspensions was 3.74 mg/L (see table below). These results demonstrate that current OECD criteria for a valid test have been satisfied.

#### Reliability:

score = 1, OECD guideline study

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Reference:	Davis, J.W. and Marty, G.T. 2004b. Primary Amyl Acetate (Mixed Isomers): Evaluation of ready biodegradability according to OECD Guideline 301D: Closed Bottle Test (unpublished study). Toxicology & Environmental Research and Consulting, Study ID 031114. The Dow Chemical Company, Midland, MI.
(b) Test material:	1-pentyl acetate (CAS 628-63-7)
Remark:	1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
Type:	aerobic
Inoculum:	other; aerated Lake Superior Harbor water (coarse filtered)
Concentration:	0.0, 0.8, 1.6, 3.2 ul/l (0.0, 0.7, 1.4, 2.8 mg/l)
Contact time:	20 days
Degradation:	50% after 10 days
Results:	biodegrades, but does not meet criteria for "readily biodegradable"
Kinetic:	K <sub>cf</sub> = 0.069 (+/- 0.014) per day t <sub>1/2</sub> = 10 days
Method other:	Standard Methods for the Examination of Water and Wastewater, 13 <sup>th</sup> Edition, 1980. American Public Health Association, NY. Biological Oxygen Demand (BOD) Technique.
Method:	Biodegradation of the test substance in coarse-filtered natural water was measured using a standard BOD method (APHA, 1980). Duluth-Superior (Lake Superior) harbor water was used as the test water. Water samples collected from the top 15 cm were coarse-filtered through 1 cm thick cotton, and aerated for 24 hours prior to testing. Measured characteristics of the course-filtered test water: pH, 7.56; conductivity, 138.2 umhos/cm; turbidity, 10.9 NTU; total solids, 131.7 mg/l; dissolved solids, 109.0 mg/l; suspended solids, 5.6 mg/l; alkalinity 40.9 mg CaCO <sub>3</sub> /l; hardness, 50.1 mg CaCO <sub>3</sub> /l; nitrite/nitrate-N, 0.15 mg/l; ammonia-N, 1.03 mg/l; and orthophosphate-P, 0.02 mg/l.  Tests were conducted in 300-ml BOD bottles containing 20 ml of water. Solutions of the test substance were prepared in duplicate with harbor water, at nominal concentrations of 0.0, 0.8, 1.6, and 3.2 ul/l. Bottles were filled to capacity, sealed, and incubated for 20 days at 21 +/- 3° C.  The dissolved oxygen (DO) concentration was measured in each bottle on days 0, 5, 10, 12 or 14, and 20 of incubation. DO measurements were made with an oxygen meter with self-stirring probe. Additional, random confirmatory DO measurements were conducted using the azide modification of the iodometric method (APHA, 1980).  The BOD values and chemical concentrations showing DO depletions were adjusted for the water blank and used to calculate mean BOD values from duplicate tests. The mean BOD values were compared to theoretical values and the percent theoretical BOD (% ThBOD) was

	calculated according to the method of Larson (1983). The order (n) of the biodegradation process was calculated by comparing the mean BOD values from two different concentrations of the test substance, and the nth order biodegradation rate constant was also determined (Barrow, 1979). The half-life of the test substance was estimated from the rate constant.
GLP:	no
Year:	1980
Test substance:	1-pentyl acetate, analytical grade, purity not specified
Result:	Amyl acetate followed a first-order biodegradation process in coarse-filtered (cf) Superior harbor water. The mean first-order rate constant (Kcf) (+/- SE) was 0.069 (+/- 0.014) per day. The half-life (cf) was 10.0 days.
	Additional tests were conducted, using a similar procedure, to determine the effect of suspended solids levels on biodegradation. Fine-filtered (ff) Superior harbor water (10-um filtered) was used as the test water. The suspended solids of the fine-filtered harbor water were 2.5 mg/l. Results indicated that the first-order rate constant was approximately doubled when fewer solids were present ( $K_{ff}/K_{cf} = 2.22$ ), while the half-life was approximately halved [ $\text{half-life (ff)}/\text{half-life (cf)} = 0.45$ ].
Reliability:	score = 2, reliable with restriction; purity of test substance not specified.
Reference:	Vaishnav, D.D. and L. Babeu. 1986. Occurrence and rates of chemical biodegradation in Superior harbor water. J. Great Lakes Res. (Internat. Assoc. Great Lakes Res.). 12(3):184-191.
(c) Test material:	1-pentyl acetate (CAS 628-63-7), analytical grade
Remark:	1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
Type:	aerobic
Inoculum:	other; natural water
Concentration:	0.0, 0.8, 1.6, 3.2 ul/l (0.0, 0.7, 1.4, 2.8 mg/l)
Contact time:	20 days
Degradation:	50% after 10 days (harbor water) 50% after 13 days (river water) 50% after 50 days (ground water)
Results:	biodegrades, but does not meet criteria for "readily biodegradable"
Kinetic:	Kcf = 0.069 per day (harbor water) Kcf = 0.054 per day (river water) Kcf = 0.014 per day (ground water)
Method other:	Standard Methods for the Examination of Water and Wastewater, 13 <sup>th</sup> Edition, 1980. American Public Health Association, NY. Biological Oxygen Demand (BOD) Technique.
GLP:	no
Method:	Biodegradation of the test substance in three types of natural water was measured using a standard BOD method (APHA, 1980). Superior Bay (Lake Superior) water (harbor water), Lester River water (river water), and underground well water (ground water) were

used as the test waters. All test waters were gravity filtered through 1 cm thick cotton, and aerated for 24 hours prior to testing.

Characteristics of each test water were measured in triplicate in accordance with Standard Methods (APHA, 1980).

Harbor water: pH, 7.56; conductivity, 138.2 umhos/cm; turbidity, 10.9 NTU; total solids, 131.7 mg/l; dissolved solids, 109.0 mg/l; suspended solids, 5.6 mg/l; alkalinity 40.9 mg CaCO<sub>3</sub>/l; hardness, 50.1 mg CaCO<sub>3</sub>/l; nitrite/nitrate-N, 0.15 mg/l; ammonia-N, 1.03 mg/l; and orthophosphate-P, 0.02 mg/l. Standard plate counts on nutrient agar gave a bacterial concentration of 310 cells/ml; 5 morphologically different colonies were observed.

River water: pH, 7.76; conductivity, 148.5 umhos/cm; turbidity, 1.19 NTU; total solids, 93.2 mg/l; dissolved solids, 80.7 mg/l; suspended solids, <1.2 mg/l; alkalinity, 51.8 mg CaCO<sub>3</sub>/l; hardness, 58.59 mg CaCO<sub>3</sub>/l; nitrite/nitrate-N, <0.05 mg/l; ammonia-N, 0.59 mg/l; and orthophosphate-P, <0.01 mg/l. Standard plate counts on nutrient agar gave a bacterial concentration of 420 cells/ml; 6 morphologically different colonies were observed.

Ground water: pH, 8.23; conductivity, 322.65 umhos/cm; turbidity, 0.76 NTU; total solids, 170.3 mg/l; dissolved solids, 156.7 mg/l; suspended solids, <1.2 mg/l; alkalinity, 122.6 mg CaCO<sub>3</sub>/l; hardness, 121.35 mg CaCO<sub>3</sub>/l; nitrite/nitrate-N, <0.05 mg/l; ammonia-N, 0.07 mg/l; and orthophosphate-P, <0.01 mg/l. Standard plate counts on nutrient agar gave a bacterial concentration of 55 cells/ml; 7 morphologically different colonies were observed.

Tests were conducted in 300-ml BOD bottles containing 20 ml of test water. Solutions of the test substance were then prepared in duplicate with each test water, at nominal concentrations of 0.0, 0.8, 1.6, and 3.2 ul/l. Bottles were filled to capacity with the respective test water, sealed, and incubated for 20 days at 21 +/- 3 deg. C.

The dissolved oxygen (DO) concentration was measured in each bottle on days 0, 5, 10, 12 or 14, and 20 of incubation. DO measurements were made with an oxygen meter with self-stirring probe. Additional, random confirmatory DO measurements were conducted using the azide modification of the iodometric method (APHA, 1980).

The BOD values and chemical concentrations showing DO depletions were adjusted for the water blank and used to calculate mean BOD values from duplicate tests. The mean BOD values were compared to theoretical values and the percent theoretical BOD (% ThBOD) was calculated according to the method of Larson (1983). The order (n) of the biodegradation process was calculated by comparing the mean BOD values from two different concentrations of the test substance,

and the nth order biodegradation rate constant was also determined (Barrow, 1979). The half-life of the test substance was estimated from the rate constant.

Results: 1-Pentyl acetate followed a first-order biodegradation process in all filtered test waters. Percent biodegradation of pentyl acetate (%ThBOD) at days 5, 10, 15, and 20 of incubation, by test water (estimated from reported biodegradation curves):

Harbor water: ~45% (5-days)  
~NA (10-days)\*  
~55% (15-days)  
~50% (20-days)

River water: ~40% (5-days)  
~45% (10-days)  
~50% (15-days)  
~48% (20-days)

Ground water: ~10% (5-days)  
~15% (10-days)  
~20% (15-days)  
~25% (20-days)

\*data Not Available for 10-day biodegradation values

Mean first-order rate constants (+ half-lives), by test water:

Harbor water: 0.069/day (10 days)

River water: 0.054/day (13 days)

Ground water: 0.014/day (50 days)

Reliability: score = 2, valid with restriction; comparable to guideline study, however purity of test material not specified

Reference: Vaishnav, D.D. and L. Babeu. 1987. Comparison of occurrence and rates of chemical biodegradation in natural waters. Bull. Environ. Contam. Toxicol. 39:237-244.

(d) Test substance: Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate

Type: aerobic

Inoculum: predominantly domestic wastewater, non-adapted

Concentration: 0, 3, 7, 10 mg/l

Degradation: 72% after 20 days

Results: readily biodegradable

Kinetic: 5 day = 64%

10 day = 76%

15 day = 67%

20 day = 72%

Method other: Standard Methods for the Examination of Water and Wastewater, 13<sup>th</sup> Edition, 1971. American Public Health Association, NY. Equivalent to OECD method 301D, Closed Bottle test measuring oxygen consumption.



Settled domestic wastewater was filtered through glass wool and then added (3 ml/bottle) as seed material to clean BOD bottles. The bottles were half filled with aerated dilution water containing recommended minerals and buffer (Standard Methods for the Examination of Water and Wastewater, 13<sup>th</sup> Edition, 1971). Dilution water was sparged with oxygen to produce an available dissolved oxygen level of 30 to 35 mg/L. The test material was added to bottles from a 0.1% stock solution to yield test concentrations of 3, 7, and 10 mg/L. Concentrations were tested in duplicate. Control blanks and glucose standards were run concurrently with each test material. Dissolved oxygen in individual bottles was monitored at 5-day intervals during the 20-day test using a commercial dissolved oxygen meter fitted with an agitated probe. When the dissolved oxygen in the test bottle dropped below 4 mg/L, the contents were re-aerated using the method described by Alsop (Alsop, G.M. 1972. BOD: control test and investigative tool. Presented at West Virginia Water Pollution Control Association, Wheeling, WV, April 5-6, 1972). Samples of water were analysed for the presence of ammonia nitrogen and organic nitrogen during the course of the test. Each nitrification sample was initially screened by an ultraviolet absorption technique using a recording spectrophotometer (Standard Methods for the Examination of Water and Wastewater, 13<sup>th</sup> Edition, 1971). Samples showing an absorbance band in the wavelength range of 160 to 240 microns were then tested colorimetrically for nitrite-nitrate content and oxygen consumed in their formation subtracted from the total sample oxygen uptake to obtain the net carbonaceous oxygen demand. Results of biodegradation tests were expressed in terms of % biodegradation.

$$\% \text{ biodegradation} = \frac{100 \times (\text{oxygen demand in test bottle} - \text{oxygen demand in blank})}{\text{initial concentration of test material} \times \text{ThOD}}$$

Theoretical oxygen demand (ThOD) is the weight ratio of oxygen required per mg test material for the complete conversion of the compound to carbon dioxide and water.

Results:

Primary amyl acetate was biodegraded in freshwater. After 5 days biodegradation was 64% of the ThOD, and after 10 days, biodegradation was 76%. The biodegradation curve for primary amyl acetate demonstrates that oxidation steadily increased from test initiation until day 10, where it reached a plateau. Based on these results, primary amyl acetate can be considered readily biodegradable. The 3- and 4-carbon structural analogs of primary amyl acetate were also readily biodegraded in freshwater (see below).

### % Biodegradation of Primary Amyl Acetate<sup>1</sup> and Structural Analogs

#### in Freshwater

Test substance (C#) <sup>2</sup>	ThOD	% Biodegradation			
		5 days	10 days	15 days	20 days
1-propyl acetate (C3) <sup>3</sup>	2.04	62	80	75	72
1-butyl acetate (C4) <sup>3</sup>	2.20	58	68	70	83
primary amyl acetate (C5)	2.33	64	76	67	72
2-methyl propyl acetate (C4) <sup>4</sup>	2.20	60	74	79	81

1: Primary Amyl Acetate is a commercial mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl butyl acetate

2: number of carbons in alcohol chain of each ester

3: 1-propyl acetate and 1-butyl acetate are the 3- and 4-carbon structural analogs of 1-pentyl acetate, the major component of Primary Amyl Acetate

4: 2-methyl propyl acetate is the 4-carbon structural analog of 2-methyl butyl acetate, the minor component of Primary Amyl Acetate

- GLP: no
- Remark: Freshwater screening study. The water solubility of primary amyl acetate was reported as 0.2 g/100 ml of solution. Measured COD was reported as 2.22 mg/mg; the theoretical oxygen demand (ThOD) was reported as 2.33 mg/mg. Study lacks information regarding purity of test materials.
- Reliability: score = 2, valid with restrictions, utilized national standard methods, however purity of test material not specified
- Reference: Price, K.S. Waggy, G.T. and Conway, R.A. 1974. Brine shrimp bioassay and seawater BOD of petrochemicals. J Water Pollut Control Fed 46, 63-77.
- (e) Test substance: Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate
- Type: aerobic
- Inoculum: sea water microorganisms, non-adapted
- Concentration: 0, 3, 7, 10 mg/l
- Degradation: 87% after 20 day
- Results: readily biodegradable
- Kinetic: 5 day = 35%  
10 day = 65%  
15 day = 69%  
20 day = 87%
- Method other: Standard Methods for the Examination of Water and Wastewater, 13<sup>th</sup> Edition, 1971. American Public Health Association, NY. Equivalent to OECD method 301D, Closed Bottle test measuring oxygen consumption

The seed used was developed in seawater taken from Lavaca Bay, Texas. This seed source was maintained by adding small amounts of settled raw wastewater every 3-4 days; evaporative loss was replaced with distilled water. Von Flack's Artificial seawater was prepared according to standard methods (Standard Methods for the Examination of Water and Wastewater, 13<sup>th</sup> Edition, 1971) and was used as dilution water for this study.

Settled wastewater seed was filtered through glass wool and then added (3 ml/bottle) as seed material to clean BOD bottles. The bottles were half filled with aerated artificial seawater. Dilution water was sparged with oxygen to produce an available dissolved oxygen level of 30 to 35 mg/L. The test material was added to bottles from a 0.1% stock solution to yield test concentrations of 3, 7, and 10 mg/L. Concentrations were tested in duplicate. Control blanks and glucose standards were run concurrently with each test material. Dissolved oxygen in individual bottles was monitored at 5-day intervals during the 20-day test using a commercial high-salinity dissolved oxygen meter fitted with an agitated probe. When the dissolved oxygen in the test bottle dropped below 4 mg/L, the contents were re-aerated using the method described by Alsop (Alsop, G.M. 1972. BOD: Control Test and Investigative Tool. Presented at West Virginia Water Pollution Control Association, Wheeling, WV, April 5-6, 1972). Samples of water were analysed for the presence of ammonia nitrogen and organic nitrogen during the course of the test. Each nitrification sample was initially screened by an ultraviolet absorption technique using a recording spectrophotometer (Standard Methods for the Examination of Water and Wastewater, 13<sup>th</sup> Edition, 1971). Samples showing an absorbance band in the wavelength range of 160 to 240 microns were then tested colorimetrically for nitrite-nitrate content and oxygen consumed in their formation subtracted from the total sample oxygen uptake to obtain the net carbonaceous oxygen demand. Results of biodegradation tests were expressed in terms of % biodegradation.

$$\% \text{ biodegradation} = \frac{100 \times (\text{oxygen demand in test bottle} - \text{oxygen demand in blank})}{\text{initial concentration of test material} \times \text{ThOD}}$$

Theoretical oxygen demand (ThOD) is the weight ratio of oxygen required per mg test material for the complete conversion of the compound to carbon dioxide and water.

Results:

Primary amyl acetate was biodegraded in artificial seawater. After 10 days, biodegradation was 65% of the ThOD. Based on these results, primary amyl acetate can be considered readily biodegradable. The 4-carbon structural analogs of primary amyl acetate were less susceptible to biodegradation (see below).

**% Biodegradation of Primary Amyl Acetate<sup>1</sup> and Structural Analogs in Artificial Seawater**

Test substance (C#) <sup>2</sup>	ThOD	% Biodegradation			
		5 days	10 days	15 days	20 days
1-propyl acetate (C3) <sup>3</sup>	2.04	50	70	70	78
1-butyl acetate (C4) <sup>3</sup>	2.20	40	52	56	61
primary amyl acetate (C5)	2.33	35	65	69	87
2-methyl propyl acetate (C4) <sup>4</sup>	2.20	23	35	37	37

1: Primary Amyl Acetate is a commercial mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl butyl acetate

2: number of carbons in alcohol chain of each ester

3: 1-propyl acetate and 1-butyl acetate are the 3- and 4-carbon structural analogs of 1-pentyl acetate, the major component of Primary Amyl Acetate

4: 2-methyl propyl acetate is the 4-carbon structural analog of 2-methyl butyl acetate, the minor component of Primary Amyl Acetate

GLP: no

Remark: Synthetic seawater screening study. The water solubility of primary amyl acetate was reported as 0.2 g/100 ml solution. Measured COD was reported as 2.22 mg/mg; the theoretical oxygen demand (ThOD) was reported as 2.33 mg/mg. Study lacks information regarding purity of test materials.

Reliability: score = 2, valid with restrictions, utilized national standard methods, however purity of test material not specified

Reference: Price, K.S. Waggy, G.T. and Conway, R.A. 1974. Brine shrimp bioassay and seawater BOD of petrochemicals. J Water Pollut Control Fed 46, 63-77.

### 3.6 BOD<sub>5</sub>, COD OR RATIO BOD<sub>5</sub>/COD

#### BOD<sub>5</sub>

(a) Critical study and preferred value (for the mixture)

Test substance: Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate

Method other: OECD Guideline No. 301D: Ready Biodegradability, Closed Bottle Test

BOD<sub>5</sub> 36.7%

GLP: yes

Test material: primary amyl acetate, purity 99.6%

Remark: Kinetic:

5 day = 36.7%

10 day = 49.4%

17 day = 51.9%

28 day = 57.1%

Domestic wastewater, secondary effluent

Reliability: score = 1, valid without restriction; GLP guideline study

Reference: Davis, J.W. and Marty, G.T. 2004. Primary Amyl Acetate (Mixed Isomers): Evaluation of ready biodegradability according to OECD Guideline 301D: Closed Bottle Test (unpublished study). Toxicology

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& Environmental Research and Consulting, Study ID 031114. The Dow Chemical Company, Midland, MI.

- (b) Test substance: Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate
- Method other: Standard Methods for the Examination of Water and Wastewater, 13<sup>th</sup> Edition, 1971. American Public Health Association, NY. Equivalent to OECD method 301D, Closed Bottle test measuring oxygen consumption
- BOD<sub>5</sub>: 64%
- GLP: no
- Test material: primary amyl acetate, purity not specified
- Remark: Kinetic:  
                   5 day = 64%  
                   10 day = 76%  
                   15 day = 67%  
                   20 day = 72%  
                   Domestic sewage organisms, non acclimated
- Reliability: score = 2, valid with restrictions, meets national standard methods, however purity of test material not specified
- Reference: Price, K.S. Waggy, G.T. and Conway, R.A. 1974. Brine shrimp bioassay and seawater BOD of petrochemicals. J Water Pollut Control Fed 46, 63-77.
- (c) Test substance: Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate
- Method other: Standard Methods for the Examination of Water and Wastewater, 13<sup>th</sup> Edition, 1971. American Public Health Association, NY. Equivalent to OECD method 301D, Closed Bottle test measuring oxygen consumption
- BOD<sub>5</sub>: 35%
- GLP: no
- Test material: primary amyl acetate, purity not specified
- Remark: Kinetic: 5 day = 35  
                   10 day = 65  
                   15 day = 69  
                   20 day = 87  
                   sea water microorganisms, non-adapted
- Reliability: score = 2, valid with restrictions, meets national standard methods, however purity of test material not specified
- Reference: Price, K.S. Waggy, G.T. and Conway, R.A. 1974. Brine shrimp bioassay and seawater BOD of petrochemicals. J Water Pollut Control Fed 46, 63-77.

**COD**

- Test substance: Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate
- Method other: measured, chromic acid reflux procedure  
 Standard Methods for the Examination of Water and Wastewater, 13<sup>th</sup> Edition, 1971. American Public Health Association, NY.

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Test material:	primary amyl acetate, purity not specified
GLP:	no
COD:	2.22 mg/mg substance
Reliability:	score = 2, valid with restrictions, utilized national standard methods, however purity of test material not specified
Reference:	Price, K.S. Waggy, G.T. and Conway, R.A. 1974. Brine shrimp bioassay and seawater BOD of petrochemicals. J Water Pollut Control Fed 46, 63-77.

**Ratio BOD<sub>5</sub>/COD:**

BOD <sub>5</sub> /COD:	
Remark:	no data
Reference:	

**ThOD**

## (a) Critical study and preferred result

Test substance:	Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate
Method other:	calculated
ThOD:	2.34 mg/mg substance
GLP:	yes
Reliability:	score = 1, reliable without restriction, guideline study
Reference:	Davis, J.W. and Marty, G.T. 2004. Primary Amyl Acetate (Mixed Isomers): Evaluation of ready biodegradability according to OECD Guideline 301D: Closed Bottle Test (unpublished study). Toxicology & Environmental Research and Consulting, Study ID 031114. The Dow Chemical Company, Midland, MI.

## (b) Test substance: Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate

Method other:	calculated
ThOD:	2.33 mg/mg substance
GLP:	no
Reliability:	score = 2, reliable with restriction; acceptable calculation method
Reference:	Price, K.S. Waggy, G.T. and Conway, R.A. 1974. Brine shrimp bioassay and seawater BOD of petrochemicals. J Water Pollut Control Fed 46, 63-77.

**3.7 BIOACCUMULATION**

## (a) Critical study and preferred result (for the mixture)

Test substance:	Primary Amyl Acetate; mixture of 65% 1-pentyl acetate and 35% 2-methyl-1-pentyl acetate.
BCF:	14.57 L/kg
Method	other: calculated, BCFWIN v2.15
Year:	2000
Remark:	BCF value of 14.57 was calculated using

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- Log BCF =  $0.77 (\log Kow) - 0.70 + cf$ , where log Kow is 2.423 and cf (correction factor) is 0.  
 Log BCF = 1.16  
 Reliability: score = 2, valid with restriction; accepted calculation method  
 Reference: BCFWIN. Version 2.15. U.S.EPA. 2000. EPI Suite software, Version 3.12. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (b) Critical study and preferred result (for major component only)  
 Test substance: 1-pentyl acetate (CAS 628-63-7)  
 Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%  
 BCF: 31 L/kg  
 Method: other (estimated)  
 Year:  
 GLP: no  
 Test substance: 1-pentyl acetate (CAS 628-63-7)  
 Remarks: Using a octanol/water partition coefficient of 2.258 (chosen by Howard, 1990), an estimated bio-concentration factor of 31 was determined using recommended regression equations. Based on this value, 1-pentyl acetate would not be expected to be significant in an aquatic ecosystem..  
 Reliability: score = 2, reliable with restriction; accepted calculation method  
 Reference: Howard, P.H. 1990. Handbook of Environmental Fate and Exposure Data for Organic Chemicals. Volume II Solvents. Chelsea, MI: Lewis Publishers, Inc.  
 Lyman, W.J. et al. 1982. Handbook of Chemical Property Estimation Methods. New York: McGraw Hill.
- (b) Test substance: 1-pentyl acetate (CAS 628-63-7)  
 Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%  
 BCF: 11.78 L/kg  
 Method: other: calculated, BCFWIN v2.15  
 Year: 2000  
 Remark: BCF value of 11.78 was calculated using  
 Log BCF =  $0.77 (\log Kow) - 0.70 + cf$ , where log Kow of 2.34 and cf (correction factor) of 0.  
 Log BCF = 1.102  
 Reliability: score = 2, valid with restriction; accepted calculation method  
 Reference: BCFWIN. Version 2.15. U.S.EPA. 2000. EPI Suite software, Version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (c) Test substance: 1-pentyl acetate (CAS 628-63-7)

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- |               |  |
|---------------|--|
| Remark:       | 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%   |
| BCF:          | 30 L/kg  |
| Method other: | estimated using regression-derived equations   |
| Year:         | 1990   |
| Remark:       | BCF value of 30 was estimated based on a log Kow of 2.3 and a water solubility of 2000 ppm at 25° C, respectively. Values chosen by Franke et al. (1994).  |
| Reference:    | Lyman, W.J. et al. 1990. Handbook of Chemical Property Estimation Methods. Environmental Behaviour of Organic Compounds. Washington, DC: American Chemical Society. <i>cited in:</i> Franke, C. et al. 1994. The assessment of bioaccumulation. Chemosphere 29: 1501-1504. |
- (d) Critical study and preferred value (for minor component only)
- |                |   |
|----------------|---|
| Test material: | 2-methyl-1-butyl acetate (CAS 624-41-9)   |
| Remark:        | 2-methyl-1-butyl acetate is the minor component of Primary Amyl Acetate, and is present in the mixture at a concentration of 35%  |
| BCF:           | 11.05 L/kg  |
| Method         | other: calculated, BCFWIN v2.15   |
| Year:          | 2000  |
| Remark:        | BCF value of 11.05 was calculated using<br>$\text{Log BCF} = 0.77 (\text{log Kow}) - 0.70 + \text{cf}$ , where log Kow of 2.26 and cf (correction factor) of 0.<br>$\text{Log BCF} = 1.043$   |
| Reliability:   | score = 2, valid with restriction; accepted calculation method  |
| Reference:     | BCFWIN. Version 2.15. U.S.EPA. 2000. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at:<br><a href="http://www.epa.gov/oppt/exposure/docs/episuite.htm">http://www.epa.gov/oppt/exposure/docs/episuite.htm</a> |

**3.8 ADDITIONAL REMARKS**

- (a) Critical study and preferred result (for the mixture)
- |                 |   |
|-----------------|---|
| Test substance: | Primary Amyl Acetate; mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate.   |
| Type:           | Henry's Law constant  |
| Method:         | Calculated using the molecular structure. Value self-selected by model for other calculations (volatilization, distribution)  |
| Result:         | $2.932 \times 10^{-4}$ atm-m <sup>3</sup> /mol  |
| GLP:            | Not applicable  |
| Reliability:    | Score=2, valid with restrictions, accepted calculation method   |
| Reference:      | HENRYWIN v3.10, U.S.EPA. 2000. EPI Suite software, version 3.12. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at:<br><a href="http://www.epa.gov/oppt/exposure/docs/episuite.htm">http://www.epa.gov/oppt/exposure/docs/episuite.htm</a> |



## 3. ENVIRONMENTAL FATE AND PATHWAYS

ID: 628-63-7, 624-41-9

DATE: 01.07.2006

- (b) Critical study and preferred value (for major component only)
- Type: Henry's Law Constant
- Test substance: 1-pentyl acetate (CAS 628-63-7)
- Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
- Method: Calculated (at 25 degree C) using water solubility 2000 mg/L, vapor pressure (3.5 mm Hg), and molecular weight 130.19 g/mol.
- Result:  $3.00\text{E-}4$  atm-m<sup>3</sup>/mol
- GLP: Not applicable
- Reliability: Score = 2, valid with restrictions, accepted calculation method
- Reference: Lyman, W.J., et al. 1982. Handbook of Chemical Property Estimation Methods. Environmental Behavior of Organic Compounds. McGraw-Hill NY.
- (c)
- Type: Henry's Law constant
- Test substance: 1-pentyl acetate (CAS 628-63-7)
- Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
- Method: other
- Result:  $3.91 \times 10^{-4}$  atm-m<sup>3</sup>/mol
- GLP: no data
- Reliability: score = 2, reliable with restriction; data from Handbook or collection of data.
- Reference: Howard, P.H. 1990. Handbook of Environmental Fate and Exposure Data for Organic Chemicals. Volume II Solvents. Chelsea, MI: Lewis Publishers, Inc.
- (d)
- Type: Henry's Law constant
- Test substance: 1-pentyl acetate (CAS 628-63-7)
- Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
- Method: other
- Result:  $3.88 \times 10^{-4}$  atm-m<sup>3</sup>/mol
- GLP: no data
- Reliability: score = 2, reliable with restriction; data from Handbook or collection of data.
- Reference: HSDB (Hazardous Substance Database), 2002. n-Amyl Acetate, CASRN 628-63-7. HSDB No. 5126. Complete update on 7/22/02.
- (e)
- Type: Henry's Law constant
- Test material: 2-methyl-1-butyl acetate (CAS 624-41-9)
- Remark: 2-methyl-1-butyl acetate is the minor component of Primary Amyl Acetate, and is present in the mixture at a concentration of 35%
- Method: Calculated (at 25 degree C) using water solubility 1070 mg/L, vapor pressure 6.36 mm Hg, and molecular weight 130.19 g/mol.
- Result:  $1.016\text{E-}3$  atm-m<sup>3</sup>/mol
- GLP: Not applicable
- Reliability: Score=2, valid with restrictions, accepted calculation method

## 3. ENVIRONMENTAL FATE AND PATHWAYS

ID: 628-63-7, 624-41-9

DATE: 01.07.2006

- Reference: Lyman, W.J., et al. 1982. Handbook of Chemical Property Estimation Methods. Environmental Behavior of Organic Compounds. McGraw-Hill NY.
- (f) Critical study and preferred result: (for minor component only)
- Type: Henry's Law constant
- Test material: 2-methyl-1-butyl acetate (CAS 624-41-9)
- Remark: 2-methyl-1-butyl acetate is the minor component of Primary Amyl Acetate, and is present in the mixture at a concentration of 35%
- Method: Calculated using the molecular structure. Value self-selected by model for other calculations (volatilization, distribution)
- Result:  $5.35\text{E-}4 \text{ atm-m}^3/\text{mol}$
- GLP: Not applicable
- Reliability: Score=2, valid with restrictions, accepted calculation method
- Reference: HENRYWIN v3.10, U.S.EPA. 2003. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (g) Test substance: 1-pentyl acetate (CAS 628-63-7)
- Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
- Remark: Waste water treatment: activated charcoal adsorbability: 0.17 g/g carbon: 88% reduction  
influent 985 mg/L, effluent 119 mg/L.
- Reference: Giusti, D.M, Conway, R.A. and Lawson, C.T. 1974. Activated carbon adsorption of petrochemicals. J Water Pollution Control Federation 46: 946-965. *cited in*: Verschueren, K. 2001. Handbook of Environmental Data on Organic Chemicals, 4<sup>th</sup> edition. New York, NY: John Wiley & Sons, Inc. p.197.

**4.0 ECOTOXICOLOGICAL DATA****4.1 ACUTE/PROLONGED TOXICITY TO FISH****(a) Critical study and preferred result (for the mixture)**

Test substance: Primary Amyl Acetate, mixture of approximately 65%  
1-pentyl acetate and 35% 2-methyl-1-butyl acetate

Type: static

Species: *Pimephales promelas* (fathead minnow)

Unit: mg/l

Exposure Period: 96 hour

NOEC:

LC50: 69

LC100:

Analyt. Monitoring: no

Method other: Standard Methods for Examination of Water and Wastewater, 13<sup>th</sup>  
Edition. 1971. American Public Health Association, NY.

Ten fish (2.5 to 5.0 cm) were exposed to each of five nominal test concentrations (50, 75, 100, 150, and 200 mg/L) and an untreated control. The volume of each test solution was 18.5 L. Dissolved oxygen in test vessels was maintained between 7.5 and 9.0 mg/L throughout the test interval by use of controlled aeration, which was initiated after the first four hours of the test. The purity of the test material was not verified. No information was provided regarding maintenance of test concentrations during the exposure interval. Water temperatures were maintained at 71 to 76 degree F. The pH of dilution water used was 7.2 to 7.6. The initial pH of the test system containing a nominal concentration of 100 mg/l 1-pentyl acetate was 7.4. Total alkalinity was measured at 30-40 mg/l as calcium carbonate; total hardness at 30-60 mg/l. LC50 values were reported as 69 mg/L (24 hr), 69 mg/L (48 hr), and 69 mg/L (96 hr). Dissolved oxygen, temperature, and pH were monitored daily. Fish were observed daily and mortality recorded.

GLP: no

Year: 1974

Test substance: primary amyl acetate, purity not specified

Results: LC50 (24hr) = 69 mg/l  
LC50 (48 hr) = 69 mg/l

Remark: Structural analogs of the components of primary amyl acetate were also tested using the same protocol. Results suggest that the short-chain esters have similar toxicity to fish (see below).

Toxicity of Primary Amyl Acetate<sup>1</sup> and Structural Analogs to Fathead Minnow

Test substance (C#) <sup>2</sup>	Initial pH	LC50 in mg/L		
		24-hr	48-hr	96-hr
1-propyl acetate (C3) <sup>3</sup>	7.4	100	100	80
1-butyl acetate (C4) <sup>3</sup>	7.0	66	66	66
primary amyl acetate (C5)	7.2-7.4	69	69	69
2-methyl propyl acetate (C4) <sup>4</sup>	7.7	71	71	62

1: Primary Amyl Acetate is a commercial mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl butyl acetate

2: number of carbons in alcohol chain of each ester

3: 1-propyl acetate and 1-butyl acetate are the 3- and 4-carbon structural analogs of 1-pentyl acetate, the major component of Primary Amyl Acetate

4: 2-methyl propyl acetate is the 4-carbon structural analog of 2-methyl butyl acetate, the minor component of Primary Amyl Acetate

Reliability: score = 2, valid with restrictions, meets national standard methods, however purity of test material not specified

Reference: Waggy, G.T. and Payne, J.R. 1974. Environmental Impact Analysis. Acute Aquatic Toxicity Testing. Project No. 910F44. Union Carbide Corporation, South Charleston Technical Center, South Charleston, WV.

- (b) Test substance: Amyl Acetate, [isomer(s) not specified]  
 Type: static  
 Species: *Semolitus atromaculatus* (creek chub)  
 Unit: mg/l  
 Exposure Period: 24 hours  
 NOEC:  
 LC0: 50  
 LC50:  
 LC100: >120  
 Analyt. Monitoring: no  
 Method other: Fish (3 to 4 inches in length) were obtained from a commercial dealer, maintained in a stock tank, and fed goldfish food every other day. Water used for control and dilution of the test material was taken from the stock tank. Stock tank water (results of analysis provided in report) was continuously aerated and the temperature was maintained between 18 and 21° C.  
 A series of tests were conducted over a range of concentrations. The purity of the test material was not verified. Test solutions were obtained by dissolving a known weight of amyl acetate in a given volume of water. Lower concentrations were prepared by dilution with stock tank water. Groups of 4 fish were placed in glass containers containing the test material in 3 liters of stock tank water. The test solution was aerated and the temperature maintained between 15 and 21° C. Stock water used to make test solutions had a pH of 8.3; total hardness (as calcium carbonate) was 98, specific conductance at 25° C was 218 micromhos, dissolved solids were 120. Control fish were exposed to stock water only. Fish were observed at frequent intervals

	over the 24 hr test period. The toxicity of primary amyl acetate was expressed as the "critical range", defined as the concentration in ppm below which there was no mortality, and above with there was 100% mortality
GLP:	no
Year:	1952
Result:	Specific toxicity data for fish at each concentration tested not described, however authors report that fish usually succumbed within 3 to 4 hours. The critical range for amyl acetate (unspecified isomers) was estimated to be between 50 and 120 ppm. The critical range is defined as the concentration in ppm below which there was no mortality, and above with there was 100% mortality.
Reliability:	score = 3, documentation insufficient for assessment
Reference:	Gillette, L.A., Miller, D.L. and Redman, H.E. 1952. Appraisal of a chemical waste problem by fish toxicities tests. Sewage Industrial Wastes 24: 1397-1401.
(c) Test material:	1-butyl acetate
Remark:	1-butyl acetate is a structural analog of 1-pentyl acetate. 1-Pentyl acetate is the major component of primary amyl acetate.
Type:	Flow-through
Species:	<i>Pimephales promelas</i> (Fathead minnow)
Exposure Period:	96 hours
Unit:	mg/L
Analytical Monitoring:	Yes
LC50:	18
EC50:	18
Method:	USEPA Methodology
Year:	1981
GLP:	No data
Test Substance:	Butyl acetate [CAS #123-86-4]; >99% pure; source: Aldrich Chemical Co., Milwaukee, WI
Remarks:	Fathead minnows used in the tests were cultured from brood stock provided by the USEPA Environmental Research Laboratory-Duluth. Adults were maintained in a flow-through system at 25° C with a 16-h light/dark photoperiod. Organisms were fed frozen adult brine shrimp ( <i>Artemia</i> sp.) Fry were fed freshly hatched brine shrimp nauplii three times daily until 24-h before test initiation. Fish were not fed during the test. Fish used in the toxicity tests were 31-32 d old and had a mean length of $21.6 \pm 2.55$ mm and a mean weight of $0.175 \pm 0.061$ g. The loading rate was 0.694 g/l. The toxicity test was conducted using a flow-through exposure regime. The tank volume was 2.8 L. The control/dilution water was either de-chlorinated laboratory water that was supplemented with minerals or filtered Lake Superior water. Total hardness was $43.0 \pm 0.00$ mg/l (as $\text{CaCO}_3$ ), alkalinity was $34.0 \pm 0.00$ mg/l (as $\text{CaCO}_3$ ), dissolved oxygen was $6.0 \pm 0.45$ mg/l, and

pH was  $7.20 \pm 0.08$ . The test temperature ranged from 24.6 to 25.4° C.

Fifty fish (2 replicates of 25 fish each) were exposed to the control/dilution water and to each of five concentrations of the test substance. Nominal (range of measured) concentrations tested were 15 (4.4-4.6), 24 (8.2-8.3), 41 (15-16), 69 (23-27), and 115 (37-39) mg/l. Test concentrations were analyzed daily by gas liquid chromatography. Mortality and abnormal signs of behaviour were recorded at 6, 12, 24, 30, 48, 49, 72, and 96 hours.

#### Results of the toxicity test on butyl acetate:

Control/dilution water: No mortality observed

15 mg/l: No mortality observed.

24 mg/l: No mortality observed.

41 mg/l: 2 dead at 72 h, 4 dead at 96 h (92% survival)

69 mg/l: 6 dead at 12 h, 30 dead at 24 h, 50 dead at 49 h (0% survival)

115 mg/l: 50 dead at 12 h (0% survival)

Authors reported that affected fish lost equilibrium prior to death. The EC/LC50 and 95% confidence limits were estimated based on measured test concentrations using the Trimmed Spearman-Kärber method.

Results: 96-h EC/LC50 and 95% confidence limits = 18 (17-19) mg/l  
 Reliability: score = 1, valid without restriction; comparable to guideline study  
 Reference: Brooke, L.T., D.J. Call, D.L. Geiger, and C.E. Northcott (ed.). 1984. Acute Toxicities of Organic Chemicals to Fathead Minnows (*Pimephales promelas*). Center for Lake Superior Environmental Studies. University of Wisconsin-Superior.

- (d) Test substance: 1-propyl acetate (CAS Reg. No. 109-60-4)  
 Remark: 1-propyl acetate is a structural analog of 1-pentyl acetate; 1-pentyl acetate is the major component of primary amyl acetate.  
 Type: Flow through  
 Species: *Pimephales promelas* (fathead minnow)  
 Exposure period: 96 hours  
 Unit: mg/l  
 Anal Monitoring: yes  
 LC50: 60  
 EC50: 60  
 Method: other (USEPA)  
 Year: 1984  
 GLP: no  
 Test substance: 1-propyl acetate, purity >99%  
 Method: Fathead minnows used in the tests were cultured from brood stock provided by the USEPA Environmental Research Laboratory-Duluth. Adults were maintained in a flow through system at 25° C with a 16-h light/dark photoperiod. Organisms were fed frozen adult brine shrimp (*Artemia* sp.) Fry were fed freshly hatched brine shrimp nauplii three times daily until 24-h before test initiation. Fish were not fed during

the test. Fish used in the toxicity tests were 30 d old and had a mean length of  $20.4 \pm 1.73$  mm and a mean weight of  $0.148 \pm 0.030$  g. The loading rate was 0.587 g/l. The toxicity test was conducted using a flow-through exposure regime. The tank volume was 6.3 L.

The control/dilution water was either dechlorinated laboratory water that was supplemented with minerals or filtered Lake Superior water. Total hardness was  $45.0 \pm 0.00$  mg/l (as  $\text{CaCO}_3$ ) and alkalinity was  $35.5 \pm 0.00$  mg/l (as  $\text{CaCO}_3$ ).

The test was initiated using 50 (30-d old) organisms, randomly distributed to each of five test concentrations, and an untreated control. The test was conducted with two replicates (25 fish per replicate) for each concentration tested and the control water. Dechlorinated tap water or Lake Superior water was used as control and dilution water. The purity of the test material and the test concentrations were analyzed by gas-liquid chromatography. Measurements of the test substance in the test concentrations were made at least four times during the exposure period.

Nominal (and range of measured) concentrations tested: 0 (0-0) mg/l, 35 (9.6-10.9) mg/l, 58 (16-17) mg/l, 97 (21-25) mg/l, 162 (48-49) mg/l, and 269 (95-105) mg/L. The test temperature ranged from 23.8 to 25.4 deg. C. The dissolved oxygen concentration was 6.4-7.2 mg/L; pH was 7.18-7.28.

Mortality and signs of abnormal behavior were recorded at 6, 12, 24, 48, 72, and 96 hours. The 96-h EC/LC50 and 95% confidence limits were calculated using mean measured data by the Trimmed Spearman-Kärber Method (Hamilton, et al. 1977).

Results:

Average corrected measured concentrations were 0, 9.7, 15.5, 22, 46, and 94.5 mg/L. The test temperature was 23.8-25.4° C. The concentration of dissolved oxygen was 6.4-7.2 mg/L; pH was 7.23 +/- 0.5 SU.

There was no control mortality. Affected fish lost equilibrium prior to death.

Results of the 96-h acute toxicity test of propyl acetate:

Control: No mortality observed

35 mg/l: No mortality observed

58 mg/l: No mortality observed

97 mg/l: No mortality observed

162 mg/l: 2 dead at 72 h, 6 dead at 49 h

269 mg/l: 15 dead at 12 h, 43 dead at 24 h, 50 dead at 48 h

All test organisms in the 269 mg/l concentration died within 48 hours of exposure to the test substance. Authors noted that affected fish lost equilibrium prior to death.

Reliability:

96 hr EC50/LC50 (95% confidence limit) = 60 (56-64) mg/L

score = 1, valid without restriction; comparable to guideline study

Reference:	Brooke, L.T., D.J. Call, D.L. Geiger, and C.E. Northcott (eds.). 1984. Acute Toxicities of Organic Chemicals to Fathead Minnows ( <i>Pimephales promelas</i> ). Center for Lake Superior Environmental Studies. University of Wisconsin-Superior.
(e) Test substance:	1-propyl acetate (CAS Reg. No. 109-60-4)
Remark:	1-propyl acetate is a structural analog of 1-pentyl acetate; 1-pentyl acetate is the major component of primary amyl acetate.
Type:	static
Species:	<i>Pimephales promelas</i> (fathead minnow)
Unit:	mg/l
Exposure Period:	96 hour
NOEC:	
LC50:	81.7
LC100:	
Analyt. Monitoring:	no
Method other:	Standard Methods for Examination of Water and Wastewater, 13 <sup>th</sup> Edition. 1971. American Public Health Association, NY.
	<p>Ten fish (2.5 to 5.0 cm) were exposed to each of five nominal test concentrations (50, 100, 200, 350, and 500 mg/L) and an untreated control. The volume of each test solution was 18.5 L. Dissolved oxygen in test vessels was maintained between 7.5 and 9.0 mg/L throughout the test interval by use of controlled aeration, which was initiated after the first four hours of the test. The purity of the test material was not verified. No information was provided regarding maintenance of test concentrations during the exposure interval. Water temperatures were maintained at 71 to 76 degree F. The pH of dilution water used was 7.2 to 7.6. The initial pH of the test system containing a nominal concentration of 100 mg/l of 1-propyl acetate was 7.4. Total alkalinity was measured at 30-40 mg/l as calcium carbonate; total hardness at 30-60 mg/l. LC50 values were reported as 100 mg/L (24 hr), 100 mg/L (48 hr), and 80 mg/L (96 hr). Dissolved oxygen, temperature, and pH were monitored daily. Fish were observed daily and mortality recorded.</p>
GLP:	no
Year:	1974
Test substance:	primary amyl acetate, purity not specified
Results:	<p>The initial LC50 values were recalculated using the Trimmed Spearman-Kärber Method (Version 1.0, 1986), based on reported response data</p> <p>24-h LC50 (95% CL) = 100 (80.3-124.5) mg/L</p> <p>48-h LC50 (95% CL) = 96.6 (72.9-127.9) mg/L</p> <p>96-h LC50 (95% CL) = 81.7 (63.6-105.0) mg/L</p>
Reliability:	score = 2, valid with restrictions, meets national standard methods, however purity of test material not specified
Reference:	Waggy, G.T. and Payne, J.R. 1974. Environmental Impact Analysis. Acute Aquatic Toxicity Testing. Project No. 910F44. Union Carbide Corporation, South Charleston Technical Center, South Charleston, WV.



- (f) Value: 11.746 mg/L  
Test substance: Primary Amyl Acetate; mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate.  
Remark: An acute fish 96-h LC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used, as was a log Kow of 2.42, water solubility 1700 mg/L, and molecular weight 130.19 g/mol. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.  
Reliability: Score=2, reliable with restrictions, calculated value  
Reference: ECOSAR model (v. 0.99h). U.S. EPA. EPI Suite Software. 2000. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (g) Value: 12.962 mg/L  
Test substance: 1-Pentyl acetate (CAS # 628-63-7; major component of Primary Amyl Acetate)  
Remark: An acute fish 96-h LC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used, as was a log Kow of 2.34, water solubility 2000 mg/L, and molecular weight 130.19 g/mol. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.  
Reliability: Score=2, reliable with restrictions, calculated value  
Reference: EcoSAR model (v. 0.99g). U.S. EPA. EPI Suite Software. 2003. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (h) Value: 14.304 mg/L  
Test substance: 2-methyl-1-butyl acetate (CAS # 624-41-9; minor component of Primary Amyl Acetate)  
Remark: An acute fish 96-h LC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used, as was a log Kow of 2.26, water solubility 1070 mg/L, and molecular weight 130.19 g/mol. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.  
Reliability: Score=2, reliable with restrictions, calculated value  
Reference: ECOSAR model (v. 0.99g), U.S.EPA. 2000. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (i) Value: 39.914 mg/L  
Test substance: 1-Propyl acetate (CAS # 109-60-4)  
Remark: An acute fish 96-h LC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used as was a log Kow of 1.23,

- melting point -92°C, water solubility 18,900 mg/L, and molecular weight 102,13 g/mol.. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.
- Reliability: Score=2, reliable with restrictions, calculated value  
Reference: EcoSAR model (v. 0.99g). EPI Suite Software. 2000. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at:  
<http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (j) Value: 22 mg/L  
Test substance: 1-Butyl acetate (CAS # 123-86-4)  
Remark: An acute fish 96-h LC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used, as was a log Kow of 1.82, melting point -77°C, water solubility 14,000 mg/L, and molecular weight 116.16 g/mol.. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.
- Reliability: Score=2, reliable with restrictions, calculated value  
Reference: EcoSAR model (v. 0.99g). EPI Suite Software. 2000. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at:  
<http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (k) Value: 23 mg/L  
Test substance: 2-methyl-1-propyl acetate (CAS # 110-19-0)  
Remark: An acute fish 96-h LC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used, as was a log Kow of 1.78, melting point -99°C, water solubility 6000 mg/L, and molecular weight 116.16 g/mol. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.
- Reliability: Score=2, reliable with restrictions, calculated value  
Reference: ECOSAR model (v. 0.99h), U.S.EPA. 2000. EPI Suite software, version 3.12. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at:  
<http://www.epa.gov/oppt/exposure/docs/episuite.htm>

## 4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

### A. *Daphnia*

- (a) Critical study and preferred result (for the mixture)  
Test substance: Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate  
Type: static  
Test: EC50 (immobilization)  
Species: *Daphnia magna* Straus, freshwater invertebrate  
Unit: mg/L  
Exposure Period: 48 hours

LC50: 40.9  
 NOEC: 3.77  
 Test substance: Primary Amyl Acetate, purity 99.6%  
 Analyt Monitoring.: yes  
 Year: 1992  
 GLP: yes  
 Methods: OECD Guideline 202, *Daphnia sp.*, Acute Immobilization Test, Part 1. 4 April 1984.

Directive 91/414/EEC, C.2 Annex I8.2.5.

USEPA, Toxic Substances Control Act Test Guidelines. Daphnid Acute Toxicity Test 40 CFR 797.1300. July 1, 1992.

The test organism, *Daphnia magna* Straus, was originally obtained from Aquatic Biosystems, Fort Collins, CO. Daphnid instars less than 24 hours old from laboratory-reared cultures were used as the test organism. Daphnid cultures were maintained under illumination (cool-white fluorescent,  $2050 \pm 350$  lux) on a 16-hour light/8-hour dark photoperiod at a temperature of  $20 \pm 2$  °C. Daphnia were fed a mixed diet of algae (*Selenastrum capricornutum*) and YCT (yeast-ceraphyll trout) trout chow five times per week. First instar daphnids (< 24-hr old) were separated from adults and older instars on the day of testing by gentle screening through a nylon mesh and metal sieve.

Laboratory water was obtained from the upper Saginaw Bay of Lake Huron; before use, water was sand-filtered, pH-adjusted with gaseous carbon dioxide, carbon-filtered, and UV-irradiated. Daphnid dilution water (DDW) was prepared by adjusting laboratory water to a hardness of about 170 mg/L (as CaCO<sub>3</sub>) before autoclaving at 250 °F and 18 psi for 30 minutes. DDW was cooled, then aerated for approximately 24 hours before use. Water quality parameters of DDW were as follows:

Hardness (mg CaCO<sub>3</sub>/L): 176  
 Alkalinity (mg CaCO<sub>3</sub>/L): 38  
 Conductivity (umhos/cm): 408  
 Residual chlorine: <10 ppb

Definitive testing was conducted in 250 ml covered glass beakers with a test solution volume of 200 ml. Test vessels were placed in an incubator thermostated at  $20 \pm 1$  °C. Temperature, pH, and dissolved oxygen were monitored and recorded for each bulk solution on Day 0 and for all spent test solutions on Day 2 (48 hours). Daphnids were not fed during the test. Daphnids were observed for immobility (inability to swim within 15 seconds after gentle agitation of the test container) after 24 and 48 hours of exposure.

Primary amyl acetate nominal test concentrations were selected based on two 48-hour static probe studies. Both studies were conducted with one replicate of 10 *Daphnia* per dose level exposed to primary amyl acetate at concentrations of 12.5, 25, 50, 100, 200 and 400 mg/L plus a water control. In the range-finding assay, analytical chemistry verification of test solutions indicated that the majority of the test

material hydrolyzed in water over the 48 hour test interval. As a result, the range-finding study was repeated and conducted under static-renewal conditions (renewal of test solutions after 24 hours).

The second range-finding study was conducted using static-renewal conditions with one replicate of 10 daphnids per dose level exposed to primary amyl acetate at concentrations of 12.5, 25, 50, 100, 200 and 400 mg/L. Immobility was observed in 100% (10/10), 80% (8/10), 50 (5/10), 90 (9/10), and 10% (1/10) of daphnids at the 400, 200, 100, 50, and 25 mg/L dose levels. No immobility was observed in among daphnids exposed to 12.5 mg/L primary amyl acetate or to the water control.

The definitive test was performed under static-renewal conditions. Twenty *Daphnia* (10 per replicate; two replicates per dose level) were exposed to nominal test concentrations of primary amyl acetate of 6.25, 12.5, 25, 50, 100, 200 and 400 mg/L plus a water control. These nominal target concentrations were equivalent to mean measured concentrations of 3.77, 7.82, 16.2, 33.3, 70.3, 145, and 289 mg primary amyl acetate/L, respectively. Test solutions were prepared in bulk on Day 0 by direct addition of the test substance to daphnid dilution water without pH adjustment and then apportioned between two replicate test vessels per dose. Daphnids were added to each test vessel within 30 minutes of solution preparation and initial test solution sampling.

Samples of the fresh bulk test solutions were analyzed on Day 0 and Day 1, and all spent control and treatment vessel solutions were analyzed on Day 1 and Day 2. Dissolved oxygen, pH, and temperature data were recorded for each fresh bulk dose solution at test initiation and at 24 hours, and for spent test solutions at 24 hours and at test termination. Samples were analyzed by gas chromatography/flame ionization detector (GC/FID) using an Agilent 6890N GC and Agilent 7683 tower autosampler (standard preparation, detector calibration, instrument conditions for GC/FID analysis described in detail in report).

Statistical analysis: Due to the biological response observed following 24 hours exposure, the USEPA Probit Program Version 1.5 was not appropriate for the statistical evaluation of the 24-hr biological data for this study. Therefore, using mean measured primary amyl acetate concentrations, the USEPA Trimmed Spearman-Kärber Program, Version 1.5 was used to calculate the 24-hour EC50 values and corresponding percent 95% confidence intervals. The Probit Program was used to calculate the 48-hour EC50 value and corresponding 95% confidence intervals. The 48-hour NOEC was determined based on biological interpretation of the data and the highest level exhibiting no *Daphnia* immobility.

## Results:

Day 0 analysis of bulk test solutions for primary amyl acetate acid demonstrated that dose solutions ranged from 87.2% to 105% of target nominal values; Day 1 renewal bulk dose test solutions had percent of target levels ranging from 96% to 116%. The average % of target for day 0 solutions was  $97.5 \pm 6.36\%$ ; the average percent of target for Day 1 renewal solutions was  $102 \pm 7.38\%$ . Analyses of spent test solutions demonstrated declines in primary amyl acetate; percent of target levels ranged from 30.8 to 43.2% for Day 1 spent solutions, and 23.8 to 47.4% in Day 2 spent solutions. Mean measured concentrations were calculated for all dose levels by averaging the Day 0 concentrations and Day 4 spent test solution concentrations (see table below). The concentration of primary amyl acetate in water control solutions was less than the lowest level quantified or less than 0.0545 mg/L.

## Results of Analysis of Test Solutions for Primary Amyl Acetate

Target Concentration (mg/L)	Concentrations (mg/L)				
	Day 0 Solution % Target <sup>1</sup>	Day 1 Spent	Day 1 Renewal	Day 2 Spent	Mean Measured <sup>2</sup>
Control	<LLQ <sup>3</sup>	<LLQ	<LLQ	<LLQ	NA <sup>4</sup>
6.25	87.2	33.0	96.2	24.8	3.77
12.5	92.8	30.8	96.0	30.7	7.82
25.0	95.6	43.2	97.2	23.8	16.2
50.0	99.4	33.0	99.6	34.2	33.3
100	98.7	31.9	105	45.4	70.3
200	104	31.8	107	47.4	145
400	105	39.3	NA-I	NA-I <sup>5</sup>	289

1: Average % of target dose on Day 0:  $97.5 \pm 6.26$ ; on Day 1:  $102 \pm 7.38$

2: Mean measured concentration = mean of Day 0 fresh solution and Day 1 spent solution concentration values

3: less than Lowest Level Quantified: 0.546 mg/ primary amyl acetate/L DDW

4: Not applicable

5: Not applicable-I: 100% immobility observed after 24 hours

pH values ranged from 7.0 – 7.6 ( $7.3 \pm 0.2$ ) at test initiation. Temperature during the test was between 20.1 and 20.9 ( $20.5 \pm 0.2$ ) °C; light intensity 1736-2300 ( $2007 \pm 219$ ) lux. Dissolved oxygen was between 7.5 – 9.4 ( $8.5 \pm 0.4$ ) mg/L. Percent oxygen saturation averaged 96% and remained greater than 84% throughout the exposure interval, based on a theoretical oxygen saturation value of 8.9 mg/L.

Observations were made for immobility in response to exposure to primary amyl acetate. At 24 hours, immobility was observed in 100% of *Daphnia* (20/20) exposed to the highest nominal dose level of 400 mg/L. At 48 hours, immobility was observed in exposed daphnids at all but the lowest test concentration (see table below).

## Biological Response to Primary Amyl Acetate Exposure

Target Concentration (mg/L)	Primary Amyl Acetate (MMC in mg/L) <sup>1</sup>	Biological Response Observed	
		24 hours	48 hours
Control	<LLQ <sup>2</sup>	20N	20N
6.25	3.77	20N	20N
12.5	7.82	19N, 1I	19N, 1I
25.0	16.2	19N, 1I	18N, 2I
50.0	33.3	19N, 1I	12N, 8I
100	70.3	14N, 6I	7N, 13I
200	145	8N, 12I	20I
400	289	20I	20I

N = normal; I = immobile.

1: Mean measured concentration

2: Less than Lowest Level Quantified: 0.546 mg/L

The NOEC was determined based on the highest dose level exhibiting no *Daphnia* immobility.

Based mean measured Primary Amyl Acetate concentrations:

The 24-hour EC50 value was 96.3 mg/L, with a 95% confidence interval of 75.1 – 123 mg/L. the Spearman-Kärber trim was 0.0%.

The 48-hour EC50 was 40.9 mg/L, with a 95% confidence interval of 31.6 – 52.9 mg/L. The Probit slope was 2.9 with a 95% confidence interval of 2.1-3.8.

The 48-hour NOEC was 3.77 mg/L and was determined based on biological interpretation of the data and the highest exposure level exhibiting no *Daphnia* immobility.

Reliability:

score = 1, valid without restriction; GLP guideline study.

Reference:

Marino, T.A., Hales, C.A., McClymont, M.S. and Yaroeh, A.M.. 2003. Primary Amyl Acetate: An Acute Toxicity Study with the Daphnid, *Daphnia magna* Straus (unpublished study). Toxicology & Environmental Research and Consulting, Study ID 031092. The Dow Chemical Company, Midland, MI.

- (b) Test substance: 1-pentyl acetate (CAS 628-63-7)  
 Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%  
 Type: static  
 Species: *Daphnia magna* (Crustacea, water flea)  
 Unit: mg/l  
 Exposure Period: 24 hours  
 NOEC:  
 LC0: 16  
 LC50: 210  
 LC100: 500  
 Analyt. Monitoring: no data  
 Method other:  
 GLP: no

Remark:	A standardized static test was conducted using 24-hr old <i>D. magna</i> . The control/dilution water was de-chlorinated tap water. The dissolved oxygen concentration was greater than saturation; the hardness was approximately 286 mg/l, the pH was 7.6-7.7. Temperature of the water ranged from 20 to 22° C. Study lacks information regarding purity of test material.
Reliability:	score = 4, not assignable, original reference in foreign language; non-standardized test interval.
Reference:	Bringmann, G. and Kuhn, R. 1977. Befunde der Schadwirkung wassergefährdender Stoffe gegen <i>Daphnia magna</i> . Z.f. Wasser-und Abwasser-Forschung. 10(Nr5/77): 161-166.
(c) Test substance:	Primary amyl acetate, concentrations of isomers not specified
Type:	static
Species:	<i>Daphnia sp.</i> (Crustacea , water flea)
Unit:	mg/L
Exposure Period:	48 hours
NOEC:	
EC0:	440
EC50:	580
EC100:	960
Analyt. Monitoring:	no data
Method other:	
GLP:	no
Test substance:	primary amyl acetate, purity not specified
Remark:	Threshold toxic effect concentration at 23° C is 440 mg/L
Reliability:	score = 4, not assignable; original reference not available
Reference:	McKee, J.E. and Wolf, H.W. 1971. Water Quality Criteria. US Public Health Service, Control Board, Pub.3-A, dated April 7, 1971. cited in: Verschueren, K. 2001. Handbook of Environmental Data on Organic Chemicals, 4 <sup>th</sup> edition. New York, NY: John Wiley & Sons, Inc. p.197.
(d) Test substance:	Primary Amyl Acetate; mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate.
Value:	42.892 mg/L
Remark:	An acute daphnid 48-h LC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used, as was a log Kow of 2.42, water solubility 1700 mg/L, and molecular weight 130.19 g/mol. . The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.
Reliability:	Score=2, reliable with restrictions, calculated value
Reference:	EcoSAR model (v. 0.99h). U.S.EPA. 2000. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <a href="http://www.epa.gov/oppt/exposure/docs/episuite.htm">http://www.epa.gov/oppt/exposure/docs/episuite.htm</a>

## 4. ECOTOXICOLOGICAL DATA

ID: 628-63-7, 624-41-9

DATE: 01.07.2006

- (e) Test substance: 1-pentyl acetate (CAS # 628-63-7; major component of Primary Amyl Acetate)  
 Value: 50.719 mg/L  
 Remark: An acute daphnid 48-h LC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used, as was a log Kow of 2.34, water solubility 2000 mg/L, and molecular weight 130.19 g/mol. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.  
 Reliability: Score=2, reliable with restrictions, calculated value  
 Reference: EcoSAR model (v. 0.99g). U.S.EPA. 2000. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (f) Value: 59.975 mg/L  
 Test substance: 2-methyl-1-butyl acetate (CAS # 624-41-9; minor component of Primary Amyl Acetate)  
 Remark: An acute daphnid 48-h EC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used, as was a log Kow of 2.26, water solubility 1070 mg/L, and molecular weight 130.19 g/mol. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.  
 Reliability: Score=2, reliable with restrictions, calculated value  
 Reference: ECOSAR model (v. 0.99g), U.S.EPA. 2000. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (g) Value: 407.26 mg/L  
 Test substance: n-propyl acetate (CAS # 109-60-4)  
 Remark: An acute daphnid 48-h EC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used, as was a log Kow of 1.23, melting point -92°C, water solubility 18,900 mg/L, and molecular weight 102.13 g/mol. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.  
 Reliability: Score=2, reliable with restrictions, calculated value  
 Reference: ECOSAR model (v. 0.99g), U.S.EPA. 2000. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (h) Value: 134.543 mg/L  
 Test substance: n-butyl acetate (CAS # 123-86-4)  
 Remark: An acute daphnid 48-h EC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used, as was a log Kow of 1.82, melting point -77°C, water solubility 14,000 mg/L, and molecular weight 116.16 g/mol. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.



- Reliability: Score=2, reliable with restrictions, calculated value  
 Reference: ECOSAR model (v. 0.99g), U.S.EPA. 2000. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (i) Value: 149.40 mg/L  
 Test substance: 2-methyl-1-propyl acetate (CAS # 110-19-0)  
 Remark: An acute daphnid 48-h EC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used, as was a log Kow of 1.78, melting point -99°C, water solubility 6000 mg/L, and molecular weight 116.16 g/mol. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.
- Reliability: Score=2, reliable with restrictions, calculated value  
 Reference: ECOSAR model (v. 0.99f), U.S.EPA. 2002. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>

**B. Other**

- (a) Critical study and preferred result  
 Test substance: Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate  
 Type: static  
 Species: *Artemia salina* (brine shrimp)  
 Unit: mg/L  
 Concentration: 0, 10, 18, 32, 56, and 100  
 Exposure Period: 24 hours  
 LC0: 10  
 LC50: 53  
 LC100:  
 Analyt. Monitoring: no data  
 Method other: Brine shrimp were hatched into 1-liter wide mouth bottles equipped with a fritted glass air diffuser. Black plastic tape was wrapped around each bottle, with a small window left open. Von Flack's Artificial seawater was prepared according to standard methods (Standard Methods for the Examination of Water and Wastewater, 13<sup>th</sup> Edition, 1971). One gram of eggs was placed into each bottle which was then filled with artificial seawater. Brine shrimp eggs were provided by Carolina Biological Supply Co, Burlington, NC. The water was aerated until shrimp were hatched, then aeration was stopped. A light source was placed at each window; viable shrimp were drawn to the light, which were then drawn off with a medicine dropper and placed in a fresh container containing artificial seawater. The shrimp suspensions were kept mixed with a magnetic stirrer. The number of shrimp per ml was determined by filtering a known volume through a gridded membrane and counting the shrimp under a low power microscope.

The range of concentrations toxic to freshly hatched brine shrimp was determined in a static screening study using shrimp suspensions with a titer of 30 to 50 shrimp per ml. Appropriate volumes of a 1% solution of test material were placed in 150 ml, wide-mouth bottles to achieve concentrations of 10,000, 1000, and 100 mg/ml. Artificial seawater was added to provide a total volume of 100 ml/bottle. The brine shrimp suspension (1 ml) was then pipetted into each bottle. Bottles were capped and incubated at ambient temperature (24.5 degree C) for 24 hr. Bottles which contained only seawater were run concurrently as controls. Sodium lauryl sulfate was run as the positive control standard.

Results of the screening test were used to establish dosage concentrations in the definitive test. Definitive testing was conducted as described above for screening tests except that primary amyl acetate was tested at concentrations of 0, 10, 18, 32, 56, and 100 mg/L. By plotting the percent survival versus test concentration (log scale), a straight-line plot is obtained. The concentration at which the line intersects with 50% survival is reported as the LC<sub>50</sub> or TL<sub>m</sub> (Threshold Median Limit).

At the end of the 24-hr exposure interval, the number of live and dead brine shrimp were counted by viewing the shrimp and by the use of a colony counter. Dead shrimp displayed no movement of phyllopodia (swimming appendages which also function as gills); unaffected shrimp displayed rapid fluttering of phyllopodia.

Year: 1974

GLP: no

Test substance: primary amyl acetate, purity not specified

Results: Results of an initial screening test indicated that primary amyl acetate had an LC<sub>50</sub> of less than 100 mg/L. Definitive testing was conducted using concentrations of 0, 10, 18, 32, 56, and 100 mg/L. The 24-hr LC<sub>50</sub> was determined to be 53 mg/L. Because this study listed the results of over 50 substances, there are no additional descriptions regarding the effect of the test material on brine shrimp behavior.

Results recorded for each concentration tested were as follows:

66% mortality at 100 mg/L

52% mortality at 56 mg/L

28% mortality at 32 mg/L

12% mortality at 18 mg/L

0 % mortality at 10 mg/L

The 24-hr LC<sub>50</sub> for the positive control, sodium lauryl sulfate, was 3.6 mg/L.

Remark: Survival data derived from graphical determination of LC<sub>50</sub> for primary amyl acetate

Reliability: score = 2, valid with restrictions; purity of test substance not specified, number of replicates not specified

Reference: Price, K.S. Waggy, G.T. and Conway, R.A. 1974. Brine

- shrimp bioassay and seawater BOD of petrochemicals. J Water Pollution Control Fed 46, 63-77.
- (b) Test substance: 1-pentyl acetate (CAS 628-63-7)  
 Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%  
 Type: static  
 Species: *Entosiphon sulcatum* (protozoa)  
 Unit: mg/L  
 Exposure Period: 72 hours  
 NOEC:  
 EC03: 226 mg/L (toxicity threshold)  
 Analyt. Monitoring: no data  
 Method other: Stock solutions of the test compound were prepared under sterile conditions and diluted to test concentrations with twice-distilled water.  
 Stock solution I: 290 mg Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O, 70 mg Mg(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O, and 40 mg KNO<sub>3</sub> dissolved in 1000 ml twice-distilled water and filtered (0.2 µm sterile membrane filter). The pH of the stock solution was adjusted to 6.9 with KOH.
- The inoculated parallel dilution series was prepared in 300-ml Erlenmeyer flasks, stoppered with metal caps. The first flask of each series contained 16 ml of test solution. Subsequent dilutions from this flask were prepared by diluting an 8 ml aliquot of preliminary pollutant dilution with 8 ml twice-distilled water. Each flask of the dilution series was inoculated to 80 ml by adding 8 ml of stock solution I, 2 ml of preliminary protozoan cultures, and 2 ml of inactivated bacterial suspension having a known extinction value.
- Flasks were cultured at 25° C for 72 hours. Before measurement, 10% of a 0.2 µm-filtered 1.1% NaNO<sub>3</sub> solution in twice-distilled water was added to each flask. The number of protozoa was determined using a cell counter. Both the counts from the highest non-toxic concentration and the counts from the lowest concentration were compared via linear regression. A 3% reduction in cell counts (EC03) was used as the threshold value indicating the onset of inhibitory action.
- Year: 1980  
 GLP: no  
 Test substance: 1-pentyl acetate (CAS 628-63-7), purity not specified  
 Results: The "toxicity threshold", defined as the concentration of 1-pentyl acetate that produced a 3% reduction in cell growth was calculated to be 226 mg/L. Structurally similar esters produced a similar response in this assay, however the branched chain isomers appeared to be slightly less toxic.

Effect of Esters on the Protozoa, *Entosiphon sulcatum*

Test substance (C#) <sup>1</sup>	Toxicity Threshold (mg/L)
1-propyl acetate (C3) <sup>2</sup>	202
2-methyl ethyl acetate (C3) <sup>3</sup>	460
1-butyl acetate (C4) <sup>2</sup>	321
2-methyl propyl acetate (C4) <sup>3</sup>	411
1-pentyl acetate (C5) <sup>4</sup>	226

1: number of carbons in alcohol chain of each acetate ester

2: 1-propyl acetate and 1-butyl acetate are the 3- and 4-carbon structural analogs of 1-pentyl acetate, the major component of Primary Amyl Acetate

3: 2-methyl ethyl acetate and 2-methyl propyl acetate is the 3- and 4-carbon structural analogs of 2-methyl butyl acetate, the minor component of Primary Amyl Acetate

4: 1-pentyl acetate is the major component of primary amyl acetate

- Remark: Summarized results for a series of studies; lacks information on concentrations tested and purity of test material
- Reliability: score = 4, not assignable due to non-standard toxicity criteria (EC03)
- Reference: Bringmann, G. and Kuhn, R. 1980a. Comparison of the toxicity thresholds of water pollutants to bacteria, algae and protozoa in the cell multiplication test. Water Research 14: 231-241.
- (c) Test substance: 1-pentyl acetate (CAS 628-63-7)
- Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
- Type: static
- Species: *Uronema parduczi* (protozoa)
- Unit: mg/L
- Exposure Period: unknown
- NOEC:
- EC0: 550
- EC50:
- EC100:
- Analyt. Monitoring: no data
- Method other:
- Year: 1980
- GLP: no
- Reliability: score = 4, not assignable due to non-standard toxicity criteria (EC03); original reference in foreign language
- Reference: Bringmann, G. and Kuhn, R. 1980b. Bestimmung der biologischen Schadwirkung wassergefährdender Stoffe gegen Protozen. II Bakterienfressende Ciliaten. Z. Wasser/Abwasser Forsch, 1: 26-31.

#### 4.3 TOXICITY TO AQUATIC PLANTS e.g. Algae

- (a) Critical study and preferred result
- Test substance: Primary Amyl Acetate
- Type: static
- Test: EC50,
- Endpoints: cell density, growth inhibition, biomass (area under growth curve)

Species:	<i>Pseudokirchneriella subcapitata</i> (formerly know as <i>Selenastrum capricornutum</i> ), freshwater green algae
Unit:	mg/L
Exposure Period:	96 hours
EC50:	313 (186-441)
EC10:	26.3
Test substance:	Primary Amyl Acetate (mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate); purity 99.6%
Analyt Monitoring.:	yes
Year:	1992
GLP:	yes
Method:	<p>OECD Guideline 201, Algal Growth Inhibition Test            EEC Directive 92/69/EEC, C.3 Algal Inhibition Test            USEPA Toxic Substances Control Act Test Guidelines. Algal Acute Toxicity Test 40 CFR 797.1500/revision of TSCA guidelines Federal Register Vol 50 No.188.</p> <p>The green alga <i>Pseudokirchneriella subcapitata</i> (formerly known as <i>Selenastrum capricornutum</i>) was maintained in the laboratory and originated from the University of Toronto Algal Collection, Toronto, Ontario, Canada. Cultures were maintained in algal assay medium (AAM) designed by Miller <i>et al.</i> for the EPA Algal Assay Bottle Test (EPA-600/9-78-018.5) in flasks under continuous cool-white fluorescent illumination of <math>4300 \pm 650</math> lux at <math>24.0 \pm 0.2^{\circ}\text{C}</math> and continuously shaken at 100 oscillations per minute.</p> <p>Algal assay medium (AAM) was prepared by adding requisite amounts of each of the macro- and micro-nutrients into de-ionized water. Test media was pH adjusted to <math>7.5 \pm 0.1</math> and stored in the dark at approximately <math>4^{\circ}\text{C}</math> prior to use. AAM was used for toxicity tests and maintenance of algal stock cultures.</p> <p>Test solutions were prepared by direct addition of the test substance to the test medium without pH adjustment. Nominal concentrations of 0, 31.3, 62.5, 125, 250, 500, and 1000 mg/L were selected based on a range-finding test. The 500 and 1000 mg/L test solutions were sonicated for 25 and 30 minutes, respectively, to facilitate ensure complete solubility. In the range-finding assay, the percent decrease in cell density across test concentrations (8.0 to 1000 mg/L) was -1 to 99% (negative percent indicates stimulation of growth).</p> <p>Test vessels were sterilized 250-mL borosilicate Erlenmeyer flasks fitted with Shimadzu closures, each containing 100 ml assay medium. Three replicates flasks were prepared for each concentration; an additional flask was not inoculated with algae and served as a control blank. At test initiation and termination, the pH was measured for each test concentration and control blank replicates. Each flask (except the fourth control blank flask) was inoculated with 0.678 mL of the algae containing approximately <math>1.0 \times 10^6</math> cells/mL, resulting in an initial cell density of approximately 10,000 cells/mL. Flasks were placed in an environmental growth chamber and maintained at <math>24 \pm 2^{\circ}\text{C}</math> under continuous illumination at <math>8000 \pm 1600</math> lux and continuously shaken at 100 oscillations/ minute. Light intensity was</p>

measured daily; temperature was monitored continuously during the test interval.

Algal cell counts were determined by electron particle counting using a Coulter Multisizer 3. Total cell counts were determined at approximately 24, 48, 72, and 96 hours. Three separate cell count reading were made per replicate. At test termination, algal cell morphology was microscopically evaluated at 20x or 40x magnification in a hemacytometer counter chamber.

Samples of the initial test solutions were analyzed for Bulk dose solutions were sampled for analytical confirmation on day 0 of the study using Agrilent 6890N gas chromatograph equipped with a flame ionization detector. Replicates were analyzed at 96 hours at test termination. Analytical standards were prepared and analyzed with each set of samples to define detector response. Relative standard deviation values derived from daily calibrations did not exceed 2%.

Statistical analysis: study endpoints were evaluated based on the mean measured Primary Amyl Acetate concentrations and are expressed in terms of algal growth (cells per ml). Endpoints analyzed were cell density, growth rate per day, and biomass (area under the growth curve). EC<sub>50</sub> values for cell density were determined by a least squares linear regression of cell density against the log of the concentration at 72 and 96 hours. The EC<sub>gr</sub>50 value for growth inhibition for the 0-72-hr and 0-96-hr exposure intervals was not calculated due to less than a 50% effect at both time points. The EC<sub>bm</sub>50 value for biomass inhibition was calculated by regression of the differences in area under the growth curves for each dose compared to the control against the log of the concentrations for 72 and 96 hours where a clear dose-response relationship was observed. Prior to evaluation of NOEC concentrations, data were tested for normality using the Shapiro-Wilk's Test and for homogeneity of variance using the Bartlett's Test. The 72- and 96-hr cell density data and the 72- and 96-hr biomass data met the assumptions of homogeneity and normality; untransformed data for these endpoints were evaluated using the Dunnett's test. The 72- and 96-hr growth rate untransformed and transformed data (log, inverse, and square root) did not meet either normality or homogeneity assumptions. Due to low replication, a suitable nonparametric test was not available; therefore the untransformed growth rate data were evaluated using the Dunnett's test. Therefore, 72- and 96-hr data for cell density, growth rate, and biomass (area under the curve) were analyzed using analysis of variance and Dunnett's test ( $\alpha = 0.05$ ) to determine NOEC values. Because no clear NOEC values could be determined for cell density or biomass, EC<sub>10</sub> values were calculated as alternatives.

Results:

Day 0 analysis of test solutions for Primary Amyl Acetate yielded percent of target values for bulk dose solutions ranging from 90.4 to 102%, with an average percent of target of  $93.7 \pm 4.26$ . Day 4 (96 hr) test solutions yielded no quantifiable concentrations of Primary Amyl Acetate. Mean measured concentrations were calculated for all dose levels by averaging the day 0 concentrations and day 4 exposure solution concentrations.

## Results of Analysis of Test Solutions for Primary Amyl Acetate

Target Concentration (mg/L)	Day 0 Solution Concentrations (mg/L)	Day 4 Solution Concentrations (mg/L)	Mean Measured Concentrations <sup>1</sup> (mg/L)
Control	<LLQ <sup>2</sup>	<LLQ	NA <sup>3</sup>
31.3	28.5	<LLQ	14.9
62.5	56.5	<LLQ	28.9
125	117	<LLQ	59.2

## Results of Analysis of Test Solutions for Primary Amyl Acetate

Target Concentration (mg/L)	Day 0 Solution Concentrations (mg/L)	Day 4 Solution Concentrations (mg/L)	Mean Measured Concentrations <sup>1</sup> (mg/L)
250	256	<LLQ	129
500	459	<LLQ	230
1000	930	<LLQ	466

1: Mean measured concentration = mean of day 0 and day 4 concentration values

2: less than Lowest Level Quantified: 2.62 mg/L

3: Not Applicable

Since day 4 measured concentrations were all less than the lowest level quantified (LLQ = 2.26 mg Primary Amyl Acetate/L AAM), a value of 1.31 mg/L (one-half the LLQ) was used in calculations as a conservative estimate of day 4 concentrations (see table above).

Temperatures measured during the test ranged between 24.4 and 24.6 °C; mean light intensity was determined to be 7659 + 408 lux. Test solution pH values ranged from 6.6 to 6.7 at test initiation, from 9.1 to 9.7 in replicates with algae at test termination, and from 6.8 to 6.9 in blank control replicates without algae at test termination (see below).

## pH Values at Test Initiation and Termination

Primary Amyl Acetate (mg/L) <sup>1</sup>	pH values		
	Day 0	Day 4	
		with algae	no algae
<LLQ <sup>2</sup>	6.7	9.5	6.8
14.9	6.6	9.7	6.9
28.9	6.7	9.6	6.9
59.2	6.7	9.7	6.8
129	6.7	9.6	6.9
230	6.7	9.5	6.9
466	6.6	9.1	6.9

1: Mean measured concentration = mean of day 0 and day 4 concentration values

Despite the increase pH, the integrity of the test was deemed unaffected, since the control performance was unaffected. At 72 and 96 hours, the control had  $169.6 \times 10^4$  and  $463.0 \times 10^4$  cells/ml, respectively, indicating an acceptable test.

#### Mean Cell Density after 72 and 96 Hours

Primary Amyl Acetate (mg/L) <sup>1</sup>	Mean Cell Density (x10 <sup>4</sup> cells/ml)			
	72 hr	% Inhibit <sup>3</sup>	96 hr	% Inhibit
<LLQ <sup>2</sup>	169.6	NA <sup>4</sup>	463.0	NA
14.9	134.0*	21	385.2*	17
28.9	141.0*	17	413.9	11
59.2	147.0*	13	421.9	9
129	115.2*	32	364.9*	21
230	84.17*	50	307.7*	34
466	22.48*	87	115.9*	75

1: Mean measured concentration = mean of day 0 and day 4 concentration values

2: less than Lowest Level Quantified: 0.2.62 mg Primary Amyl Acetate/L

3: % inhibition relative to control value at 72 or 96 hours

4: Not Applicable

\* indicates significant difference from controls;  $p \leq 0.05$  using one-tailed Dunnett's t-test on raw data

Mean biomass (area under the growth curve) values at 72 and 96 hours displayed a similar response as that observed for cell density. The mean specific growth rates at 72 and 96 hours displayed low inhibition in cultures exposed to Primary Amyl Acetate at mean measured concentrations at or below 129 mg/ml. Significant inhibition ( $p < 0.05$ ) was observed only at 230 and 466 mg/ml.

Based mean measured Primary Amyl Acetate concentrations, the 72 hour results (95% confidence intervals) were as follows:

72-hr EC<sub>25</sub> = 94.3 (<14.9-198) mg/L based on cell density

72-hr EC<sub>50</sub> = 242 (137-347) mg/L based on cell density

72-hr E<sub>b</sub>C<sub>50</sub> = 156 (30.8 - >466) mg/L based on biomass (area under the growth curve)

72-hr E<sub>r</sub>C<sub>50</sub> = >466 mg/L based on growth rate

72-hr NOEC = 129 mg/L based on growth rate; a 72-NOEC value for cell density and biomass could not be calculated. A 72-hr EC<sub>10</sub> value of 19.6 mg/L was determined based on cell density; an E<sub>b</sub>C<sub>10</sub> of 22.4 mg/L was determined based on biomass (area under the growth curve)

Based mean measured Primary Amyl Acetate concentrations, the 96 hour results (95% confidence intervals) were as follows:

96-hr EC<sub>25</sub> = 141 (17.9-265) mg/L based on cell density

96-hr EC<sub>50</sub> = 313 (186-441)mg/L based on cell density

96-hr E<sub>b</sub>C<sub>50</sub> = 235 (29.6->466) mg/L based on biomass (area under the growth curve)



		<p>96-hr <math>E_rC_{50}</math> = &gt;466 mg/L based on growth rate per day            96-hr NOEC = 129 mg/L based on growth rate; 96-hr NOEC values for cell density and biomass could not be calculated. A 96-hr <math>EC_{10}</math> value of 26.3 mg/L was determined based on cell density; an <math>E_bC_{10}</math> of 23.5 mg/L was determined based on biomass (area under the growth curve)</p>
Reliability:		score = 1; valid without restriction; GLP guideline study
Reference:		Hancock, G.A., Hales, C.A., McClymont, E.L., and Staley, J.L. 2003. Primary Amyl Acetate: Growth Inhibition Test with the Freshwater Green Algae, <i>Pseudokirchneriella subcapitata</i> (unpublished study). Toxicology & Environmental Research and Consulting, Study ID 031087. The Dow Chemical Company, Midland, MI.
(b)	Test substance:	1-pentyl acetate (CAS 628-63-7))
	Remark:	1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
	Species:	<i>Scenedesmus quadricauda</i>
	Endpoint growth rate:	biomass (growth rate)
	Unit:	mg/L
	Exposure Period:	8 days
	EC0:	
	EC3:	80
	EC50:	
	Analyt. Monitoring:	no data
	Method other:	Stock solutions of the test compound were prepared under sterile conditions and diluted to test concentrations (by a factor of 2) with twice-distilled water. The stock solution for test cultures contained 248 mg NaNO <sub>3</sub> , 19.5 mg K <sub>2</sub> HPO <sub>4</sub> , 750 mg MgSO <sub>4</sub> ·7H <sub>2</sub> O, 360 mg CaCl <sub>2</sub> ·2H <sub>2</sub> O, 30 mg C <sub>6</sub> H <sub>5</sub> FeO <sub>7</sub> ·5H <sub>2</sub> O.
		<p>The inoculated dilution series was prepared in 300-ml Erlenmeyer flasks containing 40 ml of test solution, 5 ml of stock solution, and 5 ml algal cell suspension (collected from 10-d old stock cultures). 10 ml of each dilution series was transferred into three Kapsenberg culture tubes (18 x 80 mm), stoppered with metal caps, placed on a white surface, and protected from daylight. Twelve control cultures were prepared. Tubes were maintained for 8 days under continuous artificial lighting, temperature of 27 deg. C, relative humidity of 50%, and pH of 7.0. Tubes were shaken once daily.</p> <p>Algal cell concentrations were measured turbidimetrically and expressed as the extinction of the primary light of monochromatic radiation at 578 nm for a 10 mm layer. Cell growth inhibition was graphically determined in a semi-logarithmic coordinate system. Both the highest non-toxic test concentration and the lowest toxic test concentration were plotted. A 3% reduction in cell growth was used as the threshold value indicating the onset of inhibitory action.</p>
	Year:	1976, 1978a, 1978b, 1980
	GLP:	no

Test substance:	1-pentyl acetate (CAS 628-63-7)
Remark:	Study lacks information on exposure concentrations tested, purity of test material, and response data.
Reliability:	score = 3, invalid due to non-standard exposure interval (8 days)
Reference:	Bringmann, G. and Kuhn, R. 1980a. Comparison of the toxicity thresholds of water pollutants to bacteria, algae and protozoa in the cell multiplication test. Water Research 14: 231-241.
	Bringmann, G., and Kuhn, R. 1976. Vergleichende Befunde der Schadwirkung wassergefährdender Stoffe gegen Bakterien ( <i>Pseudomonas putida</i> ) and Blaualgen ( <i>Microcystis aeruginosa</i> ). Gwf-Wasser/Abwasser 117 (9).
	Bringmann, G., and Kuhn, R. 1978a. Testing of substances for their toxicity threshold: Model organisms <i>Microcystis</i> ( <i>Diplocystis</i> ) <i>aeruginosa</i> and <i>Scenedesmus quadricauda</i> . Mitt. Internat. Verein. Limnol. 21: 275-284.
	Bringmann, G. and R. Kühn. 1978b. Grenzwerte der schadwirkung wassergefährdender stoffe gegen blualgen ( <i>Microcystis aeruginosa</i> ) und grünalgen ( <i>Scenedesmus quadricauda</i> ) im zellvermehrungshemmtest. Vom Wasser. 50:45-60.
(c) Test substance:	1-pentyl acetate (CAS 628-63-7))
Remark:	1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
Species:	<i>Microcystis aeruginosa</i>
Endpoint growth rate:	biomass
Unit:	mg/L
Exposure Period:	8 days
NOEC:	
LOEC:	
EC3:	63
EC10:	
EC50:	
Analyt. Monitoring:	no
Method other:	Closed, static test system. Stock solutions of the test compound were prepared under sterile conditions and diluted to test concentrations (by a factor of 2) with distilled water. Test cultures and controls were maintained for 8 days under continuous artificial lighting, at 27 degree C, and a relative humidity of 50%. Cultures were shaken once daily. Algal cell concentrations were measured turbidimetrically and expressed as the extinction of the primary light of the monochromatic radiation at 578 nm for a 10 nm layer. Cell growth inhibition was graphically determined in a semi-logarithmic coordinate system. The highest non-toxic concentration and the lowest toxic concentration were plotted. A 3% reduction (EC3) in cell growth was used as the value indicating onset of growth inhibition.
Year:	1976, 1978

- |                 |  |
|-----------------|--|
| GLP:            | no   |
| Test substance: | 1-pentyl acetate (CAS 628-63-7)  |
| Remark:         | Study lacks information on dilution water. Exposure concentrations not reported or measured.   |
| Reliability:    | score = 3, invalid due to non-standard exposure interval (8 days)  |
| Reference:      | Bringmann, G., and Kuhn, R. 1976. Vergleichende Befunde der Schadwirkung wassergefährdender Stoffe gegen Bakterien ( <i>Pseudomonas putida</i> ) and Blaualgen ( <i>Microcystis aeruginosa</i> ). Gwf-Wasser/Abwasser 117 (9).                       |
|                 | Bringmann, G., and Kuhn, R. 1978a. Testing of substances for their toxicity threshold: Model organisms <i>Microcystis</i> ( <i>Diplocystis</i> ) <i>aeruginosa</i> and <i>Scenedesmus quadricauda</i> . Mitt. Internat. Verein. Limnol. 21: 275-284. |
- (d) Test substance: Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate
- Value: 0.950 mg/L
- Remark: An acute algal 96-h EC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used, as was a log Kow of 2.42, water solubility 1700 mg/L, and molecular weight 130.19 g/mol. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.
- Reliability: Score=2, valid with restrictions, calculated value
- Reference: EcoSAR model (v. 0.99h). U.S.EPA. 2000. EPI Suite software, version 3.12. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (e) Test substance: 1-pentyl acetate (CAS# 628-63-7; major component of Primary Amyl Acetate)
- Value: 1.045 mg/L
- Remark: An acute algal 96-h EC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used, as was a log Kow of 2.34, water solubility 2000 mg/L, and molecular weight 130.19 g/mol. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.
- Reliability: Score=2, valid with restrictions, calculated value
- Reference: EcoSAR model (v. 0.99g). U.S.EPA. 2000. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (f) Critical study and preferred value (for this component only)
- Test substance: 2-methyl-1-butyl acetate (CAS# 624-41-9; minor component of Primary Amyl Acetate)
- Value: 1.150 mg/L
- Remark: An acute algal 96-h EC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used, as was a log Kow of 2.26,

- water solubility 1070 mg/L, and molecular weight 130.19 g/mol. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.
- Reliability: Score=2, valid with restrictions, calculated value  
Reference: ECOSAR model (v. 0.99g), U.S.EPA. 2000. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (g) Test substance: n-propyl acetate (CAS# 109-60-4)  
Value: 3.089 mg/L  
Remark: An acute algal 96-h EC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used, as was a log Kow of 1.23, melting point -92°C, water solubility 18,900 mg/L, and molecular weight 102.13 g/mol. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.
- Reliability: Score=2, valid with restrictions, calculated value  
Reference: ECOSAR model (v. 0.99g), U.S.EPA. 2000. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (h) Test substance: n-butyl acetate (CAS# 123-86-4)  
Value: 1.736 mg/L  
Remark: An acute algal 96-h EC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used, as was a log Kow of 1.82, melting point -77°C, water solubility 14,000 mg/L, and molecular weight 116.16 g/mol. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.
- Reliability: Score=2, valid with restrictions, calculated value  
Reference: ECOSAR model (v. 0.99g), U.S.EPA. 2000. EPI Suite software, version 3.11. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Available at: <http://www.epa.gov/oppt/exposure/docs/episuite.htm>
- (i) Value: 1.821mg/L  
Test substance: 2-methyl-1-propyl acetate (CAS # 110-19-0)  
Remark: An acute algal 96-h EC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used, as was a log Kow of 1.78, melting point -99°C, water solubility 6000 mg/L, and molecular weight 116.16 g/mol. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.
- Reliability: Score=2, reliable with restrictions, calculated value  
Reference: ECOSAR model (v. 0.99h), U.S.EPA. 2000. EPI Suite software, version 3.12. United States Environmental Protection Agency, Office

of Pollution Prevention and Toxics, Washington, DC. Available at:  
<http://www.epa.gov/oppt/exposure/docs/episuite.htm>

#### 4.4 TOXICITY TO BACTERIA

(a) Critical study and preferred result (for this component only)

Test substance: 1-pentyl acetate (CAS 628-63-7)  
 Remark: 1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%  
 Type: aquatic  
 Species: *Escherichia coli*  
 Unit: mg/L  
 Exposure Period: 16 hr  
 Toxicity threshold: 145  
 Analyt. Monitoring: no data  
 Method other: Cell multiplication inhibition test. Stock solutions of the test compound were prepared under sterile conditions and diluted to test concentrations (by a factor of 2) with twice-distilled water.

The inoculated 4-parallel dilution series was prepared in 300-ml Erlenmeyer flasks, stoppered with cotton-lined plastic caps. The first flask of each series contained 160 ml of test solution. Subsequent dilutions from this flask were prepared by diluting an 80 ml aliquot of preliminary pollutant dilution with 80 ml twice-distilled water. Each flask of the dilution series was inoculated to 100 ml by adding 5 ml of stock solution I, 5 ml of stock solution II, and 10 ml of bacterial cell suspension from the preliminary culture. Blank controls (not containing inoculum) were prepared by adding 5 ml of stock solution I, 5 ml of stock solution II, and 10 ml saline solution (0.50 g NaCl/l sterile, twice-distilled water).

Stock solution I: 20.0 g D(+) glucose, 4.240 g NaNO<sub>3</sub>, 2.40 g K<sub>2</sub>HPO<sub>4</sub>, 1.20 g KH<sub>2</sub>PO<sub>4</sub>, and 30 ml trace elements solution dissolved in 500 ml twice-distilled water and sterilized for 30 minutes.

Stock solution II: 0.20 g FeSO<sub>4</sub>·7H<sub>2</sub>O and 4.00 g MgSO<sub>4</sub>·7H<sub>2</sub>O dissolved in 1000 ml sterile, twice-distilled water.

Flasks were cultured at 25 deg. C for 16 hours. At test termination, the concentration of bacterial cells was measured turbidimetrically and expressed as the extinction of the primary light of monochromatic radiation at 436 nm for a 10 mm layer. Bacterial cell growth inhibition was graphically determined. Both the highest non-toxic test concentration and the lowest toxic test concentration were plotted. A 3% reduction in cell growth was used as the threshold value indicating the onset of inhibitory action.

Year: 1980  
 GLP: no

Remark:	Study lacks information on concentrations tested, purity of test material, and response data.
Reliability:	score = 2, valid with restrictions; purity of test material not specified, range of concentrations tested not specified.
Reference:	Bringmann, G. and Kuhn, R. 1980a. Comparison of the toxicity thresholds of water pollutants to bacteria, algae and protozoa in the cell multiplication test. Water Research 14: 231-241.
(b) Test substance:	1-pentyl acetate (CAS 628-63-7)
Remark:	1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
Type:	aquatic
Species:	<i>Pseudomonas putida</i>
Unit:	mg/L
Exposure Period:	16 hour
EC3:	145
EC10:	
EC50:	
Analyt. Monitoring:	no data
Method other:	<p>toxicity threshold - cell multiplication test. Stock solutions of the test compound were prepared under sterile conditions and diluted to test concentrations (by a factor of 2) with twice-distilled water.</p> <p>The inoculated 4-parallel dilution series was prepared in 300-ml Erlenmeyer flasks, stoppered with cotton-lined plastic caps. The first flask of each series contained 160 ml of test solution. Subsequent dilutions from this flask were prepared by diluting an 80 ml aliquot of preliminary pollutant dilution with 80 ml twice-distilled water. Each flask of the dilution series was inoculated to 100 ml by adding 5 ml of stock solution I, 5 ml of stock solution II, and 10 ml of bacterial cell suspension from the preliminary culture. Blank controls (not containing inoculum) were prepared by adding 5 ml of stock solution I, 5 ml of stock solution II, and 10 ml saline solution (0.50 g NaCl/l sterile, twice-distilled water).</p> <p>Stock solution I: 20.0 g D(+) glucose, 4.240 g NaNO<sub>3</sub>, 2.40 g K<sub>2</sub>HPO<sub>4</sub>, 1.20 g KH<sub>2</sub>PO<sub>4</sub>, and 30 ml trace elements solution dissolved in 500 ml twice-distilled water and sterilized for 30 minutes.</p> <p>Stock solution II: 0.20 g FeSO<sub>4</sub>·7H<sub>2</sub>O and 4.00 g MgSO<sub>4</sub>·7H<sub>2</sub>O dissolved in 1000 ml sterile, twice-distilled water.</p> <p>Flasks were cultured at 25 deg. C for 16 hours. At test termination, the concentration of bacterial cells was measured turbidimetrically and expressed as the extinction of the primary light of monochromatic radiation at 436 nm for a 10 mm layer. Bacterial cell growth inhibition was graphically determined. Both the highest non-toxic test concentration and the lowest toxic test concentration were plotted. A 3% reduction in cell growth was used as the threshold value indicating the onset of inhibitory action.</p>

Year: 1980  
 GLP: no  
 Remark: Study lacks information on concentrations tested, purity of test material, and response data.  
 Results: The "toxicity threshold", defined as the concentration of test substance that produced a 3% reduction in cell growth, was calculated to be 145 mg/L for 1-pentyl acetate. Structurally similar esters produced a similar response in this study.

Effect of Esters on the Bacteria, *Pseudomonas putida*

Test substance (C#) <sup>1</sup>	Toxicity Threshold (mg/L)
1-propyl acetate (C3) <sup>2</sup>	170
2-methyl ethyl acetate (C3) <sup>3</sup>	190
1-butyl acetate (C4) <sup>2</sup>	115
2-methyl propyl acetate (C4) <sup>3</sup>	200
1-pentyl acetate (C5) <sup>4</sup>	145

1: number of carbons in alcohol chain of each ester

2: 1-propyl acetate and 1-butyl acetate are the 3- and 4-carbon structural analogs of 1-pentyl acetate, the major component of Primary Amyl Acetate

3: 2-methyl ethyl acetate and 2-methyl propyl acetate is the 3- and 4-carbon structural analogs of 2-methyl butyl acetate, the minor component of Primary Amyl Acetate

4: 1-pentyl acetate is the major component of primary amyl acetate

Reliability: score = 2, valid with restrictions; purity of test material not specified, range of concentrations tested not specified.  
 Reference: Bringmann, G. and Kuhn, R. 1980a. Comparison of the toxicity thresholds of water pollutants to bacteria, algae and protozoa in the cell multiplication test. Water Research 14: 231-241.

#### 4.5 CHRONIC TOXICITY TO AQUATIC ORGANISMS

##### 4.5.1 CHRONIC TOXICITY TO FISH

No data available

##### 4.5.2. CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

No data available

#### 4.6 TOXICITY TO TERRESTRIAL ORGANISMS

No data available

##### 4.6.1 TOXICITY TO SOIL DWELLING ORGANISMS

No data available

##### 4.6.2 TOXICITY TO TERRESTRIAL PLANTS

No data available

**4.6.3 TOXICITY TO OTHER NON MAMMALIAN TERRESTRIAL SPECIES (INCLUDING AVIAN)**

No data available

**4.7 BIOLOGICAL EFFECTS MONITORING (INCLUDING BIOMAGNIFICATION)**

No data available

**4.8 BIOTRANSFORMATION AND KINETICS**

No data available

**4.9 ADDITIONAL REMARKS**

No data available



**5.0 TOXICITY****5.1 ACUTE TOXICITY****5.1.1 ACUTE ORAL TOXICITY****(a) Critical study and preferred value for males**

Test substance: Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate

Type: LD50

Species/strain: rat/Hilltop-Wistar (male)

Value: 16.0 ml/kg (14,064 mg/kg), killed 2 of 5

Method: Male Hilltop-Wistar rats (200-300 grams) received 4.0, 8.0, or 16.0 ml/kg (3,516, 7,032, or 14,064 mg/kg) of primary amyl acetate in a single dose by stomach intubation. The rats were fasted overnight prior to dosing. Animal weights were recorded at 0, 7 and 14 days. The group size at each dose level was 5 animals/group. Animals were observed for clinical signs of toxicity and survival immediately after dosing, and daily thereafter during a 14-day observation interval. The LD50 value was calculated by the moving average method (Thompson, 1947) after the animals had been observed for 14 days. Survivors were sacrificed on day 14. A gross pathology exam was conducted on animals found dead or at sacrifice.

Year: 1983

GLP: no

Test substance: primary amyl acetate, purity >98%

Remark: Two of the 5 male rats receiving primary amyl acetate at the maximum peroral dose of 16 ml/kg (14,064 mg/kg) died after one day. Survivors recovered at two days. Signs of toxicity included sluggishness, depressed respiration, unsteady gait, lacrimation, and prostration. No mortality and no signs of toxicity were observed at the lower dosages. Weight gain in surviving animals from all groups was normal. Survivors were sacrificed on day 14. At necropsy, a blanched liver and discolored fur was observed in one rat dying on study. Survivors at all dosages had no remarkable gross lesions.

Reliability: score = 1, comparable to guideline study

Reference: Myers, R.C. 1983. Union Carbide Corporation (unpublished report), Primary Amyl Acetate, Acute Toxicity and Primary Irritancy Studies. Bushy Run Research Center, Project Report 46-30, April 27, 1983.

**(b) Critical study and preferred value for females**

Test substance: Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate

Type: LD50

Species/strain: rat/Hilltop-Wistar (female)

Value: 14.0 (12.1-16.3) ml/kg [12,306 (10,636-14,428) mg/kg]

Method: Female Hilltop-Wistar rats (200-300 grams) received 4.0, 8.0, 11.3 or 16.0 ml/kg (3516, 7032, 9933, or 14,064 mg/kg) of primary amyl

		acetate in a single dose by stomach intubation. The rats were fasted overnight prior to dosing. Animal weights were recorded at 0, 7 and 14 days. The group size at each dose level was 5 animals/group. Animals were observed for clinical signs of toxicity and survival immediately after dosing, and daily thereafter during a 14-day observation interval. The LD50 value was calculated by the moving average method (Thompson, 1947) after the animals had been observed for 14 days. A gross pathology exam was conducted on animals found dead or at sacrifice.
	Year:	1983
	GLP:	no
	Test substance:	primary amyl acetate, purity >98%
	Remark:	The LD50 for females was 14 ml/kg (12,306 mg/kg) with a 95% confidence limit of 12.1 to 16.3 ml/kg (10,636-14,428 mg/kg). Mortality was observed in the 16 ml/kg group. Survivors recovered at one day. Signs of toxicity included sluggishness, depressed respiration, unsteady gait, lacrimation, and prostration. There was no mortality at the lower dosages, and sluggishness was the only sign of toxicity observed. Weight gain in surviving animals from all groups was normal. At necropsy, blanched livers and discolored fur was observed in rats dying on study. Among surviving animals, red mottled lungs were observed in rats that received 11.3 ml/kg. Survivors at the lower dosages had no remarkable gross lesions.
	Reliability:	score = 1, comparable to guideline study
	Reference:	Myers, R.C. 1983. Union Carbide Corporation (unpublished report), Primary Amyl Acetate, Acute Toxicity and Primary Irritancy Studies. Bushy Run Research Center, Project Report 46-30, April 27, 1983.
(c)	Test substance:	Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate
	Type:	LD50
	Species/strain:	rat/Hilltop-Wistar (female)
	Sex:	
	Value:	19.7 ml/kg (17,250 mg/kg)
	Method other:	Groups of five non-fasted female Hilltop-Wistar rats received primary amyl acetate by gavage at dosages which increased by a factor of 2 in a geometric series. Animals were weighed prior to dosing, and observed for 14 days after dosing. Animals were observed for clinical signs of toxicity and survival immediately after dosing, and daily thereafter during a 14-day observation interval. The LD50 value was calculated by the moving average method (Thompson, 1947) after the animals had been observed for 14 days for survival.
	Year:	1982
	GLP:	no
	Test substance:	primary amyl acetate, purity not specified
	Reliability:	score = 2, reliable with restriction; purity of test substance not specified, range of concentrations tested not specified; necropsy results not described
	Reference:	Myers, R.C. 1982. Union Carbide Corporation (unpublished report), Toxicity and Irritation Assay Results of Some Food, Drug or

Cosmetic Product Chemicals. Bushy Run Research Center, Project Report 45-194, December 13, 1982.

### 5.1.2 ACUTE INHALATION TOXICITY

(a) Critical study and preferred value

Test substance: Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate  
Type: LC50  
Species/strain: rat/Hilltop-Wistar (male and female)  
Exposure Time: 6 hours  
Value: 3693 (+/- 257) ppm killed 1 of 5 males  
3628 (+/- 330) ppm killed 0 of 5 females  
Method: Hilltop-Wistar rats, weighing between 200 and 300 g, were exposed to substantially saturated vapor for 6 hours. Vapor was generated by enclosing the test material in a sealed chamber for 18 hours. Oxygen was added, as needed, for static exposures to maintain a chamber oxygen content of approximately 20%. The mean (+/- SD) chamber concentrations for males and females were 3693 (+/- 257) ppm and 3628 (+/- 330) ppm, respectively. These static concentrations converted to mg/l are 19.7 and 19.3 mg/l, respectively.  
Year: 1983  
GLP: no  
Test substance: primary amyl acetate, purity >98%  
Remarks: Five male and five female rats were exposed to substantially saturated vapours for a 6-hour period. All animals demonstrated signs of toxicity including breathing difficulties, immobility, and impaired reflexes. Rats also displayed abnormal righting reflex, abnormal toe and tail pinch reflex, and periocular wetness immediately following exposure. One male died; the only gross pathological finding was redness of the lungs. All surviving rats appeared normal the day following exposure and throughout the remaining 14-day post-exposure observation interval. At necropsy, surviving animals had no remarkable gross lesions.  
Reliability: score = 1, comparable to guideline study  
Reference: Myers, R.C. 1983. Union Carbide Corporation (unpublished report), Primary Amyl Acetate, Acute Toxicity and Primary Irritancy Studies. Bushy Run Research Center, Project Report 46-30, April 27, 1983.

(b) Test substance: Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate  
Type: LC50  
Species/strain: rat/Hilltop-Wistar (male and female)  
Exposure Time: 4 hours  
Value: 976 (+/- 13) ppm killed 0 of 5 males, and 0 of 5 females  
Method: Hilltop-Wistar rats, weighing between 200 and 300 g, were exposed to dynamically-generated primary amyl acetate vapor for 4 hours. Vapor was generated by metering the liquid test material from a piston pump into a heated glass evaporator (Carpenter et al, 1975). Chamber concentrations were analyzed by gas chromatography at approximately once hour intervals. The mean chamber concentration was 976 (+/- 13) ppm, or 5.2 mg/L.

Test substance:	primary amyl acetate, purity >98%
Remark:	Animals exposed to dynamically-generated vapor appeared normal during and after exposure. All rats displayed a decrease in body weight gain on post-exposure day 1, but returned to pre-exposure weights by day post-exposure day 3. All surviving rats appeared normal throughout the remaining 14-day post-exposure observation interval. At necropsy, surviving animals had no remarkable gross lesions.
Reliability:	score = 1, comparable to guideline study
Reference:	Myers, R.C. 1983. Union Carbide Corporation (unpublished report), Primary Amyl Acetate, Acute Toxicity and Primary Irritancy Studies. Bushy Run Research Center, Project Report 46-30, April 27, 1983.
(c) Test substance:	Amyl Acetate, isomer not specified
Type:	Single-exposure acute neurotoxicity test in mice via whole-body inhalation exposure.
Species/strain:	mouse/Swiss (male)
Concentration:	0, 500, 1000, 2000, 4000 ppm
Exposure interval:	20 minutes
Method:	<p>Four groups of adult 8 male CFW Swiss albino mice (27-40 g) were exposed to amyl acetate vapour at target concentrations of 0, 500, 1000, 2000 or 4000 ppm. Exposures were conducted using a static system consisting of a sealed 29L cylindrical glass jar with an acrylic lid. The lid was equipped with injection ports and a fan which projected into the chamber above a 15 cm<sup>2</sup> stainless steel mesh platform. During testing, one mouse was placed in the bottom of the chamber, the lid replaced, and a measured amount of test material was injected onto filter paper located on the wire mesh platform. The fan was turned on which volatilised the test material. Vapor concentrations were confirmed by single-wavelength infrared (IR) spectrometry (Miran 1A, Foxboro Analytical). Mean concentrations were within <math>\pm 3\%</math> of the nominal concentration within 2.5 min after injection and remained within <math>\pm 2\%</math> of the target dose throughout the exposure. Each animal was exposed only once to a single test substance concentration.</p> <p>Sham exposures (air only) in static chambers were conducted once per day for five days prior to actual exposure. During each sham exposure, motor activity was monitored for 30 minutes. Motor activity was measured via two sets of photocells that bisected the static exposure chamber. Interruption of the photobeams were counted. Prior to exposure, mice were trained on the inverted screen test. Each mouse was required to climb to the top of an inverted screen within 10 seconds during three consecutive training tests. The 10-second cutoff was maintained during testing.</p> <p>Mice were observed during exposure. Measurement of motor activity was initiated immediately after activation of the chamber fan and continued during the entire 20-minute exposure interval. Stimulus reactivity and open field measurements were conducted using a functional observational battery (FOB) test (Bowen, S.E. et al. 1996. Neurotoxicol. Teratol. 18: 577-585) which consisted of 21</p>

different measures assessing five domains of behavioral/physiological responses: CNS activity, CNS excitability, autonomic effects, muscle tone/equilibrium, and sensorimotor reactivity. During the last 2 minutes of exposure, mice were scored on posture, arousal, rearing, clonic movements, tonic movements, palpebral closure, gait, and gait abnormalities. After exposure, each mouse was removed from the static chamber and evaluated for ease of removal and handling reactivity. Piloerection, righting reflex, forelimb grip strength, inverted screen test, landing foot splay, approach response, mobility, and response to audible stimulus, touch, and tail pinch were also evaluated.

Data analysis: concentration-effect curves for motor activity data were analysed using analysis of variance (ANOVA) and Turkey post hoc comparisons ( $p \leq 0.05$ ). The procedure for FOB data analysis was similar to a method used by the U.S.EPA (Creason, 1989; Tilson and Moser, 1992), with modification for between-subject data versus repeated measurements. Continuous and count measures were analysed using separate General Linear Model (GLM) procedures (SAS Institute, Cary, NC). Turkey post hoc tests were used to specify differences from control revealed by the overall analysis. Categorical data were analysed with CATMOD (SAS Institute), a procedure designed to provide a model of ANOVA for categorical data. When appropriate, frequencies of behaviors were compared to control frequencies. In addition, analyses were conducted on each domain of solvent effects by performing CATMOD procedures (Moser, 1991).

Creason, J.P. 1989. Data evaluation and statistical analysis of functional observational battery data using a linear models approach. *J. Am. Coll. Toxicol.* 8: 157-169.

Tilson, H.A. and Moser, V.C. 1992. Comparison of screening approaches. *Neurotoxicology* 13: 1-14.

Moser, V.C. 1991. Applications of a neurobehavioral screening battery. *J. Am. Coll. Toxicol.* 10: 661-669.

Results: No mortality occurred during or after exposures. No seizures or biphasic activity were observed at any concentration. No changes in motor activity was observed. During exposure, mice exposed to 4000 ppm amyl acetate exhibited decreased rearing; recovery was rapid and rearing frequency was normal within 4 minutes after termination of exposure. Mice also displayed increased palpebral closure when exposed to 4000 ppm, possibly related to the irritant characteristic of the test material. After exposure, mice exposed to 4000 and 2000 ppm displayed decrease arousal, increased handling reactivity, and clonic movements. Mice from the 4000 ppm group also displayed increased reactivity to an auditory stimulus and to pain (tail pinch). The majority of effects noted were limited to mice exposed to 4000 ppm. These results suggest that exposure to amyl acetate at concentrations of 2000 ppm or greater can produce acute transient CNS effects.

Reliability: score = 2, valid with restriction; isomer composition and purity of test Substance not specified

Reference: Bowen, S.E. and Balster, R.L. 1997. A comparison of the acute behavioural effects of inhaled amyl, ethyl, and butyl acetate in mice.

## Fundamental and Applied Toxicology 35: 189-196.

- (d) Test substance: Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate  
 Type: other, concentrated vapor inhalation  
 Species: rat, male  
 Exposure time: 4 hr and 8 hr  
 Value: approximately 5200 ppm  
 Method: other  
 Year: 1962  
 GLP: no  
 Test substance: primary amyl acetate, purity not specified  
 Remarks: Groups of 6 rats were exposed to substantially saturated vapor of primary amyl acetate (approximately 5200 ppm) for 4 or 8 hours. The concentrated vapor (approaching saturation) was produced by passing air through a fritted disc gas washing bottle containing the test substance. The resultant vapor was then flushed into an inhalation chamber. The nominal concentration produced was estimated from the amount of test substance removed from the bottle; and confirmation by analysis was not performed. Mortality was 6/6 for rats exposed for 8 hr, 0/6 for rats exposed for 4 hr.  
 Reliability: score = 2, valid with restriction; data from handbook or collection of data, purity of test material not specified.  
 Reference: Smyth, H.F. Jr, Carpenter, C.P., West, C.S., Pozzani, U.C., and Striegel, J.A. 1962. Range-finding toxicity data: List VI . Industrial Hygiene Journal 23: 95-107.

**5.1.3 ACUTE DERMAL TOXICITY**

- (a) Critical study and preferred value for males (score = 1)  
 Test substance: Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate  
 Type: LD50  
 Species: rabbit (male)  
 Strain: New Zealand White  
 Value: 9.51 (5.36 – 16.9) ml/kg [8359 (4711-14855) mg/kg]  
 Method: Male New Zealand White rabbits (2-3 kg) received dermal administration of 4.0, 8.0, or 16.0 ml/kg (3516, 7032, or 14064 mg/kg) of primary amyl acetate for 24 hours under occluded conditions on the clipped, intact skin of the trunk. Animals were immobilised during the 24-hr exposure period. After the exposure period ended, excess material was removed to prevent oral ingestion. Animals were observed for clinical signs of toxicity and mortality immediately after dosing, during the 24-hr exposure interval, at 24 hours, and daily during the 14-day observation interval. Skin reactions were observed at one hour post-dosing, and 7 and 14 days post-dosing. The group size at each dose level was 5 animals/group. Animal weights were recorded at day 0, 7, and 14 days. The LD50 value was calculated by the moving average method (Thompson, 1947) after the animals had been observed for 14 days for clinical signs and survival. Survivors were sacrificed at

	day 14. A gross pathology exam was conducted on animals found dead and at sacrifice.
Year	1983
GLP:	no
Test substance:	primary amyl acetate, purity >98%
Remark:	Local irritant signs at the application site included erythema, ecchymosis, desquamation, and scab formation. Signs of toxicity were observed at the high dose levels and included discomfort as evidence from intense struggling after application, followed by decreased activity and prostration prior to death. All deaths occurred between 1 and 7 days after application. Survivors recovered at 5 days. At necropsy, several rabbits had livers with light-coloured foci and red intestines. There were no remarkable gross lesions in survivors sacrificed after the 14 day observation interval.
Reliability:	score = 1, comparable to guideline study
Reference:	Myers, R.C. 1983. Union Carbide Corporation (unpublished report), Primary Amyl Acetate, Acute Toxicity and Primary Irritancy Studies. Bushy Run Research Center, Project Report 46-30, April 27, 1983.
(b)	Critical study and preferred value for females (score = 1)
Test substance:	Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate
Type:	LD50
Species:	rabbit (Female)
Strain:	New Zealand White
Value:	>16,000 ml/kg (>14,080 mg/kg)
Method:	A group of 5 female New Zealand White rabbits (2-3 kg) received dermal administration of 16.0 ml/kg (14080 mg/kg) of primary amyl acetate for 24 hours under occluded conditions on the clipped, intact skin of the trunk. Animals were immobilised during the 24-hr exposure period. After the exposure period ended, excess material was removed to prevent oral ingestion. Animals were observed for clinical signs of toxicity and mortality immediately after dosing, during the 24-hr exposure interval, at 24 hours, and daily during the 14-day observation interval. Skin reactions were observed at one hour post-dosing, and 7 and 14 days post-dosing. Animal weights were recorded at day 0, 7, and 14 days. The LD50 value was calculated by the moving average method (Thompson, 1947) after the animals had been observed for 14 days for clinical signs and survival. Survivors were sacrificed at day 14. A gross pathology exam was conducted on animals found dead and at sacrifice.
Year	1983
GLP:	no
Test substance:	primary amyl acetate, purity >98%
Remark:	Local irritant signs at the application site included erythema, ecchymosis, desquamation, and scab formation. Signs of toxicity were limited to signs of discomfort immediately after application. One animal died on day 2 after exposure; gross pathology revealed red lungs. There were no remarkable gross lesions in survivors sacrificed after the 14 day observation interval.

Reliability:	score = 1, comparable to guideline study
Reference:	Myers, R.C. Union Carbide Corporation (unpublished report), Primary Amyl Acetate, Acute Toxicity and Primary Irritancy Studies. Bushy Run Research Center, Project Report 46-30, April 27, 1983.
(c) Test substance:	Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate
Type:	LD50
Species:	rabbit (male)
Strain:	New Zealand White
Value:	>20,000 ml/kg (>17,580 mg/kg)
Method:	A group of 4 male New Zealand White rabbits (2.5-3.5 kg) received dermal administration of 20.0 ml/kg (17580 mg/kg) of primary amyl acetate for 24 hours under occluded conditions on the clipped, intact skin of the trunk. Animals were immobilized during the 24-hr exposure period. Animals were observed for skin reactions and signs of toxicity during a 14-day post-exposure observation interval. The LD50 value was calculated by the moving average method (Thompson, 1947) after the animals had been observed for 14 days for clinical signs and survival. Survivors were sacrificed at day 14.
Year:	1962
GLP:	no
Test substance:	primary amyl acetate, purity not specified
Reliability:	score = 2, valid with restriction; data from handbook or collection of data, purity of test material not specified.
Reference:	Smyth, H.F. Jr, Carpenter, C.P., West, C.S., Pozzani, U.C., and Striegel, J.A. 1962. Range-finding toxicity data: List VI . Industrial Hygiene Journal 23: 95-107.

#### 5.1.4 ACUTE TOXICITY, OTHER ROUTES OF ADMINISTRATION

(a) Critical study and preferred value	
Test substance:	Primary Amyl Acetate, mixture of isomers not specified
Type:	Intra-peritoneal (IP) injection
Species:	Guinea pig (male)
Value:	1500 mg/kg killed 3 of 4
GLP:	no
Test substance:	primary amyl acetate, purity not specified
Remarks:	Undiluted primary amyl acetate was administered by intraperitoneal injection to mature male guinea pigs at a dose of either 750 mg/kg or 1500 mg/kg to two groups consisting of four animals each. After 24 hours, blood was collected and animals sacrificed. Serum ornithine carbamyltransferase (SOCT) activities were measured and the livers examined.
Results:	In the 1500 mg/kg group, 3 of 4 animals died prior to sacrifice. SCOT activity in animals in the 750 mg/kg group was elevated to an average of 10.2 IU; the average control serum OCT activity was 2.02 IU. Microscopic examination of treated animals did not reveal necrosis, however, lipid deposition was considered moderate.



## 5. TOXICITY

ID: 628-63-7,624-41-9

DATE: 01.07.2006

Remark:	Limited study designed to assess the effect of high doses of solvents on the liver. The authors conclude that primary amyl acetate has a relatively low order of hepatotoxicity.
Reliability:	score = 2, valid with restriction; purity of test substance not specified.
Reference:	Divincenzo, G.D. and Krasavage, W.J. 1974. Serum ornithine carbamyl transferase as a liver response test for exposure to organic solvents. Am. Ind. Hyg. J. 35: 21-29.

**5.2 CORROSIVENESS/IRRITATION****5.2.1 SKIN IRRITATION/CORROSION**

## (a) Critical study and preferred value

Test substance:	Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate
Species:	Rabbit
Strain:	New Zealand White
Result:	moderately irritating
Classification:	Irritating
Method other:	Three male and three female New Zealand White rabbits were treated with 0.5 ml of undiluted primary amyl acetate for a 4-hour period. The dosage was applied to the clipped, intact skin under a gauze patch and was covered with impervious sheeting. The animals were restrained for the 4-hour exposure period. Excess liquid was removed at the end of the exposure period. Skin reactions were scored by the Draize method at one hour and at 1, 2, 3, 7, 10 and 14 days after exposure.
Year:	1983
GLP:	no
Test substance:	primary amyl acetate, purity >98%
Remark:	When applied to the skin of six rabbit, under semi-occluded conditions for a period of 4 hours, 0.5 ml of undiluted primary amyl acetate produced well-defined erythema on all animals and slight edema on all but one animal. Seven days following dosing, erythema was still evident in two animals while desquamation (flaking of the out skin layer) was present on all animals. These data indicate that under occluded conditions, primary amyl acetate may produce a moderate to severe local irritation of the skin, possibly accompanied by dermatitis due to the defatting properties of primary amyl acetate.
Reliability:	score = 1, valid without restriction; comparable to guideline study.
Reference:	Myers, R.C. 1983. Union Carbide Corporation (unpublished report), Primary Amyl Acetate, Acute Toxicity and Primary Irritancy Studies. Bushy Run Research Center, Project Report 46-30, April 27, 1983.

- (b) Test substance: Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate
- Species: Rabbit
- Strain: New Zealand White
- Result: slightly irritating
- Classification:

Method other:	Five albino rabbits were treated with 0.01 ml of undiluted primary amyl acetate for a 24-hour period. The dosage was applied to the clipped, intact skin and left uncovered. Skin reactions were scored by the Draize method at the end of the 24-hour exposure interval.
Year:	1962
GLP:	no
Test substance:	primary amyl acetate, purity not specified
Remark:	When applied to the skin of six rabbit for a period of 24 hours, 0.01 ml of primary amyl acetate produced capillary injection (Grade 3 on a Draize scale of 0 to 10). These data indicate that under non-occluded conditions, undiluted primary amyl acetate may produce a slight local irritation of the skin.
Reliability:	score = 2, valid with restriction; data from handbook or collection of data, purity of test material not specified.
Reference:	Smyth, H.F. Jr, Carpenter, C.P., West, C.S., Pozzani, U.C., and Striegel, J.A. 1962. Range-finding toxicity data: List VI . Industrial Hygiene Journal 23: 95-107.

### 5.2.2 EYE IRRITATION/CORROSION

(a) Critical study and preferred value (score = 1)

Test substance:	Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate
Species:	rabbit
Strain:	New Zealand White
Result:	moderately irritating
Classification:	
Method:	Three male and three female New Zealand White rabbits were dosed with volumes of 0.1 or 0.01 ml of undiluted primary amyl acetate. The dose was instilled into the lower conjunctival sac of 1 eye per animal. The eyelid was held together for one second; eyes were not rinsed. Six eyes were dosed per test volume. The eyes were scored by the Draize method at one and four hours, and 1, 2, 3, and 7 days after dosing. Fluorescein (2%) staining was used to determine corneal injury prior to dosing and at 1 day after dosing.
Year:	1983
GLP:	no
Test substance:	primary amyl acetate, purity >98%
Remark:	Instillation of 0.1 ml of primary amyl acetate did not cause corneal injury or iritis in any of 6 eyes. Moderate conjunctival irritation was noted in all six animals and persisted for as long as three days. No irritation was noted 7 days after dosing. Instillation of 0.01ml of primary amyl acetate did not cause corneal injury or iritis in any of 6 eyes. Mild conjunctival irritation was noted at one hour; 5 of 6 eyes were normal after 24 hours. One eye had slight conjunctival irritation after 24 hours, which persisted through day 3. These data indicate that primary amyl acetate is a moderate eye irritant.
Reliability:	score = 1, valid without restriction; comparable to guideline study

Reference:	Myers, R.C. Union Carbide Corporation (unpublished report), Primary Amyl Acetate, Acute Toxicity and Primary Irritancy Studies. Bushy Run Research Center, Project Report 46-30, April 27, 1983.
(b) Test substance:	Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate
Species:	rabbit
Strain:	New Zealand White
Result:	moderately irritating
Classification:	
Method :	A flooding volume of 0.5 ml was instilled directly into the rabbit eye; eyes were not rinsed. Eye injury was recorded in a 10-grade series (Grade 1 negligible injury), based upon the degree of corneal injury. See: Carpenter and Smyth, 1946. Chemical Burns of the Rabbit Cornea. American J. Ophthalmology 29: 1363.
Year:	1962
GLP:	no
Test substance:	primary amyl acetate, purity not specified
Remark	Flooding the eye with 0.5 ml primary amyl acetate resulted in Grade 2 corneal injury. These results indicate that primary amyl acetate causes minimal corneal injury.
Reliability:	score = 2, valid with restriction; data from handbook or collection of data, purity of test material not specified.
Reference:	Smyth, H.F. Jr, Carpenter, C.P., West, C.S., Pozzani, U.C., and Striegel, J.A. 1962. Range-finding toxicity data: List VI . Industrial Hygiene Journal 23: 95-107.

### 5.2.3 RESPIRATORY IRRITATION

(a) Test substance:	1-pentyl acetate, purity not specified
Remark:	1-pentyl acetate is the major component of primary amyl acetate.
Type:	Respiratory irritation (RD50) test
Species:	mouse
Result:	RD50 = 1531 ppm
Method:	See Kane, Barrow, and Alarie. 1979. A short-term test to predict acceptable levels of exposure to airborne sensory irritants. Amer. Ind. Hyg. Assoc. J. 40: 207-229. Mice were head-only exposed to amyl acetate for a 10-minute interval. It is unclear if mice were exposed to vapor, aerosol, or a combination of both. Decreases in respiratory rate were recorded and the RD50 (concentration that elicited a 50% decrease in respiratory rate) was calculated.
Year:	1979
GLP:	no data
Test substance:	1-pentyl acetate, purity not specified
Result:	Mice exposed to pentyl acetate vapors displayed a steep concentration-response relationship. There was an approximately 25% decrease in respiratory rate when mice were exposed to 700 ppm; at 2000 ppm, there was an approximately 72% decrease (values derived from concentration-response figure). The RD50 was

	determined to be 1531 ppm, with a 95% confidence interval of 1295-1902 ppm.
Remark:	The authors suggest that there is perfect correlation between the inhibition of respiration in animals and complaints of eye, nose, and throat irritation in humans. Results from RD50 tests can be used to make reasonable predictions for effects in humans. The authors conclude that a reasonable exposure limit for humans for amyl acetate, based on the RD50 test results, is 48 ppm.
Reliability:	score = 4, not assignable; documentation insufficient for assessment, composition and purity of test material not specified.
Reference:	Alarie, Y. 1981. Dose response analysis in animal studies: prediction of human responses. Environ. Health Perspect.42:9-13.

### 5.3 SKIN SENSITISATION

#### (a) Critical study and preferred value

Test substance:	Primary amyl acetate, described as a commercial blend of 65% 1-pentyl acetate and 35% 2-methyl butyl acetate), purity not specified
Type:	Patch-Test
Species:	human
Result:	not sensitising
Classification:	
Method:	A repeat-insult patch test of 20% primary amyl acetate was conducted using a panel of 211 human subjects. A total of 197 subjects completed the study. A vehicle control, 75% ethanol and 25% diethyl phthalate, was included in the study. A volume of 0.3 ml of the test material was applied to a Webril patch and allowed to volatilise for 15 minutes prior to applying the patch to the skin site. The patch remained on the skin for 24 hours. The sites were evaluated, and fresh patches applied three times per week for three weeks. The sites were evaluated 48 and 96 hours after application throughout the treatment period. Following a 10-15 day non-treatment interval, the subjects were given a challenge patch using the same dosages and contact period used in the induction phase of the study
Year:	1987
GLP:	no data
Test substance:	primary amyl acetate, described as a commercial blend of 65% 1-pentyl acetate and 35% 2-methyl butyl acetate), purity not specified
Remark:	No evidence of delayed contact hypersensitivity was observed and no adverse reactions were observed during the entire exposure period to amyl acetate.
Reliability:	score = 2, valid with restriction; data from Handbook or collection of data, composition and purity of test material not specified
Reference:	CTFA. 1987a. Submission of unpublished data by CTFA. Repeat insult patch test on amyl and isoamyl acetate. Available for review: Director, Cosmetic Ingredient Review, 1110 Vermont Ave., N.W. Suite 810, Washington, DC 20005. <i>cited in:</i> Elder, R.L. (ed). 1988. Safety Assessment of Cosmetic Ingredients. Final Report on the

- Safety Assessment of Amyl Acetate and Isoamyl acetate. J. American College of Toxicology 7: 705-719.
- (b) Test substance: amyl acetate, described as a commercial blend of 65% 1-pentyl acetate and 35% 2-methyl butyl acetate), purity not specified
- Type: photosensitization test
- Species: human
- Result: not sensitising
- Classification:
- Method: A human photoallergy and primary phototoxicity test on 20% amyl acetate was conducted using a panel of 25 human subjects. During the induction period the treatment sites were evaluated and fresh patches applied two times per week for three weeks. UV-B irradiation (26-32 mW/GW<sup>2</sup>) was also applied during the biweekly evaluation periods; subjects also received approximately 4 J UV-A. Following a 2-week non-treatment interval, the potential for photosensitization was evaluated by exposing the skin sites to UV-A, 16-20 J/cm<sup>3</sup>. A separate non-UV-exposed site was evaluated for contact sensitisation.
- GLP: no data
- Test substance: primary amyl acetate, purity not specified
- Remark: Amyl acetate did not produce a phototoxic or photoallergenic response in any of the subjects tested.
- Reliability: score = 2, valid with restriction; data from Handbook or collection of data, composition and purity of test material not specified
- Reference: CTFA. 1987b. Submission of unpublished data by CTFA. Clinical photo-toxicity and photosensitization test results. Available for review: Director, Cosmetic Ingredient Review, 1110 Vermont Ave., N.W. Suite 810, Washington, DC 20005. *cited in*: Elder, R.L. (ed). 1988. Safety Assessment of Cosmetic Ingredients. Final Report on the Safety Assessment of Amyl Acetate and Isoamyl acetate. J. American College of Toxicology 7: 705-719.
- (c) Test substance: Primary amyl acetate, described as a commercial blend of 65% 1-pentyl
- acetate and 35% 2-methyl butyl acetate), purity not specified
- Type: photosensitization test
- Species: human
- Result: not sensitising
- Classification:
- Method: A human photoallergy and primary phototoxicity test on 30% amyl acetate was conducted using a panel of 25 human subjects; 23 completed the study. The test material, 0.3 ml, was applied to separate areas of the back under occlusive Webril patches for 24 hours. The sites to be evaluated for phototoxicity were irradiated with 16-20 J/cm<sup>2</sup> of UV-A (ultraviolet A) light within 10 minutes of patch removal. The sites were evaluated at 1, 24, 48, and 72 hr post-irradiation. A separate non-UV-exposed site was evaluated for irritation.
- GLP: no data
- Test substance: amyl acetate, described as a commercial blend of 65% 1-pentyl acetate and 35% 2-methyl butyl acetate, purity not specified

Remark:	Amyl acetate did not produce a phototoxic or primary irritation response in any of the subjects tested.
Reliability:	score = 2, valid with restriction; data from Handbook or collection of data, composition and purity of test material not specified
Reference:	CTFA. 1987b. Submission of unpublished data by CTFA. Clinical photo-toxicity and photosensitization test results. Available for review: Director, Cosmetic Ingredient Review, 1110 Vermont Ave., N.W. Suite 810, Washington, DC 20005. <i>cited in:</i> Elder, R.L. (ed). 1988. Safety Assessment of Cosmetic Ingredients. Final Report on the Safety Assessment of Amyl Acetate and Isoamyl acetate. J. American College of Toxicology 7: 705-719.
(d) Test substance:	primary amyl acetate, mixture of 60% 1-pentyl acetate 35% 2-methyl-1-butyl acetate 5% 3-methyl-1-butyl acetate
Type:	Guinea pig maximization test
Species:	guinea pig
Strain:	Hartley albino
Result:	ambiguous
Classification:	
Method:	Procedures used were based on the methods described in: Magnusson, B. and Kligman, A.M. 1969. The identification of contact allergens by animal assay. The guinea pig maximization test. J. Investigative Dermatology 52: 268-276. <i>and</i> Magnusson, B. and Kligman, A.M. 1970. Allergic contact dermatitis in the guinea pig. Identification of contact allergens. Springfield, IL: Charles C. Thomas.
	<u>Induction:</u> Intradermal induction injections were made into sites in the clipped shoulder skin in each of 10 male and 10 female guinea pigs. 2 sites: 0.1 ml of a 50% emulsion of FCA (Freund's Complete Adjuvant) in water 2 sites: 0.1 ml undiluted primary amyl acetate 2 sites: 0.1 ml of a 5% solution of primary amyl acetate in FCA
	Epicutaneous induction, performed 7 days after intradermal injections, was conducted by applying 2 x 4 cm filter paper pads soaked in the test material to the re-clipped shoulder skin of guinea pigs. A 10% solution of sodium lauryl sulfate (SLS) was applied 24 hr before to provoke a mild inflammatory response. The pads soaked with test material were covered with impermeable plastic and covered with an elastic adhesive bandage. Patches were removed after 48 hr and the skin wiped to removed excess material.
	<u>Challenge:</u> Epicutaneous challenge at 2 weeks was conducted on a 5 cm square area of the clipped flank skin. A 2 cm square piece of filter paper was saturated with the test material, placed on the skin, and covered as described above. After 24 hr, the patches were removed,

and observations for dermal inflammation performed at 24 and 48 hr after removal of the challenge patch.

Positive Control: 2,4,-Dinitrochlorobenzene (DNCB) was used as a positive control in 10 guinea pigs (5 male, 5 female). Intradermal induction challenge consisted of 2 injections per animal of:

50% aqueous emulsion of FCA

0.1% DNCB in 50% FCA emulsion

0.1% DNCB in propylene glycol

Epicutaneous induction was administered 7 days later, with 2 cm square pieces of filter paper saturated with 0.1% DNCB in 80% ethanol which remained on the skin under an occlusive dressing for 48 hours. Challenge was by occluded contact with 2 cm square pieces of filter paper saturated with 0.1% DNCB in 80% ethanol which remained on the skin under an occlusive dressing for 24 hours. Observations for local dermal inflammation were made at 24- and 48-hours after removal of the challenge patch.

Irritation Control: A control group of 10 guinea pigs (5 male, 5 female) received challenge-only patches to distinguish between any primary irritant response and inflammation due to sensitisation. Each animal received undiluted primary amyl acetate on one flank and 0.1% DNCB in 80% on the opposite flank. Observations for local dermal reactions were made at 24- and 48-hours after removal of the challenge patches.

Evaluation of response: Inspection and observations for local inflammation were made at 24- and 48-hours after removal of the challenge patches. Observations were made for erythema, edema, necrosis, and eschar. Erythema was scored as no reaction, barely perceptible (usually non-confluent), slight (usually confluent), moderate, and severe. Erythema and edema greater than that seen in irritant controls was considered evidence of an allergic response.

Year: 1983

GLP: yes

Test substance: primary amyl acetate, confirmed as a mixture of 60% 1-pentyl acetate 35% 2-methyl-1-butyl acetate, and 5% 3-methyl-1-butyl acetate

Results: Pre-testing for irritancy in 6 guinea pigs demonstrated that 24-hour occluded contact with undiluted primary amyl acetate did not produce an irritant response when examined at 24- and 48-hours after removal of the patches. Following intradermal injection of 0.1 ml primary amyl acetate, only focal necrosis was observed.

Among irritant control animals in the definitive test, however, half the animals (5 of 10) displayed an irritant response to primary amyl acetate at 24 hours; 4 animals displayed very slight (barely perceptible) erythema, and 1 animal displayed slight edema. All animals were normal at 48 hours.

Following challenge with primary amyl acetate, 17 of 20 animals displayed barely perceptible erythema, and 4 of 20 displayed edema after 24 hours. After 48 hours, only 2 animals displayed minimal erythema.

Among irritant control animals that received DNCB, only 2 of 10 displayed barely perceptible erythema at 24 hours. In contrast, 24 hours after removal of the DNCB challenge patches, 10 of 10 positive animals displayed edema, and 10 of 10 displayed slight to severe erythema.

Remarks: Most (17 of 20) animals treated with primary amyl acetate displayed barely perceptible erythema at 24 hours; one exhibited a dermal score of 1 and 4 exhibited edema. However, 5 of the 10 irritation control animals also exhibited slight dermal erythema at 24 hours. At 48 hours, only two test animals had minimal erythema and no irritation was noted in control animals. Although it is possible that primary amyl acetate may possess a very slight sensitisation potential, the response observed is considered more indicative of transient irritation than sensitisation.

Reliability: score = 1, valid without restriction; comparable to guideline study  
Reference: Auletta, C.S. 1983. Union Carbide Corporation (unpublished report). Guinea Pig Maximization Test Primary Amyl Acetate and Reactive Polymer Solution (3-JWTX-46). Bio/dynamics Inc., Project No. 4128-82, February 17, 1983. *and* Ballantyne, B. and Auletta, C.S. 1986. The sensitising potential of primary amyl acetate in the guinea pig. *Veterinary and Human Toxicology* 28: 213-215.

#### 5.4 REPEATED DOSE TOXICITY

##### (a) Critical study and preferred result

Test substance: Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate

Species: rat

Strain: Fischer 344

Sex: male and female

Route of Admin: inhalation

Exposure Period: 14 weeks

Freq. of Treatment: 6 hours/day, 5 days per week

Post Exposure

Observation Period: 4 weeks

Doses: 0, 100, 300, 500 ppm

Control Group: yes

NOAEL: 500 ppm

LOAEL: NA

Method: Equivalent to OECD 413

Male and female 344 rats (55 days old at test initiation) were assigned to 4 groups, 20 per sex per group, and exposed to primary amyl acetate at target concentrations of 0, 100, 300, and 500 ppm for 6



hours per day, 5 days per week, for 14 weeks. Animals were observed prior to, during, and after each exposure; on non-exposure days, animals were observed daily. Recovery animals were observed daily. After the final exposure, half the exposed animals (10 rats per sex per exposure group) were sacrificed; the remaining animals (recovery group, 10 rats per sex per exposure group) were held for a one-month observation period and then sacrificed.

All rats were housed separately by sex and test group. During exposures, animals were housed two per cage, separated by sex and test group in stainless steel wire-mesh cages. Cage positions were rotated weekly in a predetermined pattern within each chamber to compensate for any possible but undetected variation in chamber conditions. Food and water was withheld during exposure but available *ad libitum* at all other times. Animals were exposed in 1330 L stainless steel and glass inhalation chambers for 6 hours per day. Metering of the liquid primary amyl acetate into a heat glass evaporator generated vapours of the test material. The resultant vapor was carried into the chamber by a countercurrent air stream that entered at the bottom of the evaporator. Chamber concentrations were analysed approximately once per hour using a Perkin-Elmer Model 3920B gas chromatograph equipped with a flame ionisation detector. The test material was determined to have a purity of 98.97% at test initiation, and a purity of 98.7% at termination. Average chamber concentrations were within 1 to 3% of the target concentrations.

All animals were acclimated to the exposure chambers (exposed to air only) for two days prior to initiation to the test article exposure regimen. Animals were observed prior to, during, and following each exposure for signs of toxicity. Ophthalmic examinations were conducted prior to first exposure, and at sacrifice. Body weights of all animals were recorded prior to exposure, weekly during exposure, and the day following the final exposure. Body weights for animals held during the post-exposure recovery period were recorded weekly and just prior to sacrifice. Serum chemistry and haematological evaluations were performed on blood samples collected from all rats on the day of sacrifice.

Ten rats per sex per exposure group were individually housed in round polycarbonate metabolism cages; food and water consumption was measured for approximately 14-16 hours following 68 (females) or 67 (males) exposures. Urine was collected while rats were housed in metabolism cages, food and water was available *ad libitum*. Food and water consumption was not measured for recovery rats.

Upon sacrifice, gross examinations were performed and selected tissues saved; the brain, liver, kidneys, spleen, lung, and testes (males only) from all animals were weighed at sacrifice. Histological evaluation was performed on selected tissues (male reproductive organs, spleen, lung, nasal turbinates, thymus, urinary bladder, adrenals, brain, parathyroids, heart, kidneys, larynx, thyroid, pituitary

gland, gastrocnemius muscle, liver, sciatic nerve, and trachea) from animals in the high dose (500 ppm) exposure and control groups.

Statistics: Results of quantitative continuous variables were intercompared among the three exposure groups and one control group by use of Bartlett's homogeneity of variance, analysis of variance (ANOVA), and Duncan's multiple range tests. The fiducial limit of 0.05 (two-tailed) was used as the critical level of significance for all comparisons.

Year: 1985

GLP: yes

Test substance: Primary Amyl Acetate, purity 98.97%

Remark: Lower body weight gains were observed for all groups of males exposed to primary amyl acetate throughout most of the 14-week exposure interval. The lack of a concentration-response effect, and the lack of exposure-related effects on other parameters evaluated, suggests that the decrease in body weight gain is not an exposure-related effect, but rather the result of a control group of males whose mean body weight gain was greater than normally expected. All three male exposure groups exhibited body weight gains that were comparable to control group historical controls, while the control group males from this study had greater body weight gains than historical controls.

Results: There were no clinical signs of toxicity in any of the exposure groups and no unscheduled deaths during the study. Among females, there were no exposure-related effects observed on body weight gain or absolute body weight during the study. The 100 ppm females had significantly higher body weight gains and/or absolute body weights at various intervals during the exposure period, but not during the recovery period. Among males, all exposure groups displayed similar, slight decreases in body weight gains. Body weight gains for males exposed to 100, 300, and 500 ppm were reduced by 9%, 11%, and 10%, respectively, relative to concurrent controls, but not when compared to historical control values. The body weight gains in exposed animals were comparable to historical controls for the laboratory, however, body weight gains in concurrent control males were abnormally large and were increased by 13% relative to historical controls. The apparent decreased weight gain in treated males is not a treatment-related effect, but is caused by control body weight gains that were larger than normal, since the effect was not intensified with increasing concentration, and the weight gain of exposed animals was comparable to that of the historical controls for the laboratory. There was no statistical difference between control and exposed groups in body weight gain in animals allowed to recover for 4 weeks after the final exposure. Exposure had no effect on food or water consumption in male and female exposure groups. There were no treatment related differences for any of the clinical chemistry, haematology, or urinalysis parameters evaluated. No unusual observations were noted in the appearance of internal organs. The absolute weight of the liver and testes in all male exposure groups

	<p>were significantly decreased; this effect was unrelated to concentration and was not considered to be biologically significant. There were significantly increased absolute brain and spleen weight in all female exposure groups, and increased relative brain weights in males in the 100 and 500 ppm exposure groups. Microscopic examination of all organs and tissues, and in particular those for which weight changes were observed, did not reveal any indication of injury. There were no differences in organ weights in animals at the end of the 4-week post-exposure recovery period.</p>
Reliability:	score = 1, valid without restriction; comparable to guideline study
Reference:	Klonne, D.R. 1985. Union Carbide Corporation (unpublished report). Primary Amyl Acetate Acute Fourteen Week Inhalation Vapour Study on Rats. Bushy Run Research Center, Project Report 48-14, October 8, 1985.
(b) Test substance:	Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate
Species:	rat
Strain:	Sprague-Dawley
Sex:	male and female
Route of admin:	inhalation
Exposure period:	13 weeks
Freq. of Treatment:	6 hours/day, 4-5 days per week
Doses:	0, 300, 600, and 1200 ppm
Control group:	yes
NOAEL:	1200 ppm
LOAEL:	NA
Method:	<p>Four groups of male and female Sprague-Dawley CD rats were exposed to primary amyl acetate vapour by inhalation at target concentrations of 0, 300, 600 or 1200 ppm. Animals were exposed for 13 consecutive weeks, 6 hours per day, 5 days per week except during weeks 4, 8 and 13 when animals were exposed for 4 days to accommodate the schedule for behavioral evaluations. Animals received at least 65 exposures. The control and 1200 ppm groups contained 15 rats per sex. The additional 5 rats per sex were included in the control and 1200 ppm groups for use during a possible post-exposure recovery period. Animals were examined before and after each exposure and a complete detailed clinical examination was performed weekly on each animals. During each exposure, animals were observed for overt signs of reaction to the test atmospheres. Body weights and food consumption were measured weekly. Individual animals were examined for possible changes in behaviour using automated motor activity measurements and a functional observational battery (FOB) during pre-study and once during each of weeks 4, 8 and 13.</p> <p>For motor activity evaluations, an automated apparatus assessed total activity during the 60-minute test session and for each successive 10-</p>

minute interval within the session. The apparatus counted photocell interruptions in a figure-8 enclosure (San Diego Instruments).

Statistical analysis of motor activity: If analysis of covariance (ANCOVA) with pre-exposure motor activity as a covariate were significant ( $\alpha \leq 0.050$ , then t-tests ( $\alpha \leq 0.05$ ) determined which treatment groups differed from controls for the affected day.

Functional observational battery observations were conducted accorded to Moser and co-workers (Fund. Appl. Toxicol. 11; 189-206, 1988) and assessed autonomic, CNS excitability, neuromuscular response, sensory-motor response, and other behaviors in exposed and control rats. Each rat was evaluated in three situations: 1) passive examination while rat was inside a cage to which it had been acclimated for 10-30 min; 2) examination during handling; 3) in an open field arena (see table below).

Forelimb and hindlimb grip strength were measured as the force exerted at the moment the rat released a grid or bar while its body was drawn away from a calibrated device (Chatillon strain gauge). Landing foot splay was determined as the distance between ink or paint impressions made by the rear feet after releasing the rat from a height of 30 cm. Other FOB parameters were scored according to a simple scale (absent, diminished, normal, excessive). Testers were blind with respect to treatment of animals.

Parameters Examined in Functional Observational Battery

Autonomic	CNS Excitability	Neuromuscular	Sensory- Motor	Other
lacrimation	ease of handling	gait characteristics	response to sharp sound	bizaare behavior
salivation	muscle tone	coordination		diarrhea
pupil constriction	reactivity to handler	surface to air righting	Response to toe or tail pinch	exophthalmia
defecation	tremors/ convulsions	forelimb grip strength		labored breathing
piloerection	arousal level	hindlimb grip strength		change in body weight
palpebral closure	vocalization	rear foot splay		

Statistical analysis of FOB: Continuous FOB variables (body weight, grip strength, foot splay) were analysed with ANOVA or ANCOVA using pre-test values as covariates, with t-tests or Dunnett's multiple comparisons to examine differences between treatment and the control group for specific time points.

Following completion of treatment, 5 rats per sex per group were perfused *in situ* with fixative and those animals in the control and 1200 ppm groups underwent a microscopic examination of nervous system tissues. A gross examination of respiratory tract tissues was

	performed on an additional 5 rats per sex per group and these tissues were retained for possible future microscopic examination. The remaining animals, including those designated for a possible post-exposure recovery group were euthanized and discarded.
Year:	1997
GLP:	yes
Test substance:	primary amyl acetate, purity 99.7%, mixture of 64.2% 1-pentyl acetate and 35.5% 2-methyl-1-butyl acetate
Results:	There were no mortalities and exposure to up to 1200 ppm primary amyl acetate did not result in overt clinical signs of toxicity or changes in body weight and food consumption. Transient subtle decreases in activity during exposure were noted for the 600 and 1200 ppm groups during the first two weeks of exposure; decreased activity was not observed immediately after exposure.
	There were no changes in brain weight or length in males or females. A statistically significant decrease in brain width for the 600 ppm females was attributed to intergroup variation as the difference between the groups was very small and the values for females from the control and 1200 ppm groups were comparable. There were no treatment related microscopic lesions in the brains of males or females in the 1200 ppm group and there were no gross or histopathological lesions observed in the nervous system that were attributable to treatment.
	FOB evaluations, automated motor activity measurements, and neuropathological examination did not reveal any treatment-related effects. The NOAEL for subchronic neurotoxicity for this study was 1200 ppm based on a lack of cumulative neurotoxicity following repeated exposure.
Reliability:	score = 1, valid without restriction; comparable to guideline study
Reference:	Beyrouthy, P. A 1997. 13-Week Inhalation Neurotoxicity Study by Whole- Body Exposure of Amyl Acetate Vapour in the Albino Rat. ClinTrial BioResearch Project, Report No. 97429, Bio-Research Laboratories, Ltd., Senneville, Quebec, September 25, 1997.
(c) Test substance:	Primary Amyl Acetate, mixture 68% 1-pentyl acetate and 32% 2-methyl-1-butyl acetate
Species:	rat
Strain:	Fischer 344
Sex:	male and female
Route of Admin:	inhalation
Exposure Period:	9 days
Freq. of Treatment:	6 h/day, 5 days/week
Post Exposure	
Observation Period:	none
Doses:	0, 300, 600, or 1200 ppm
Control Group:	yes
NOAEL:	600 ppm
LOAEL:	1200 ppm

**Method:** Male and female Fischer 344 rats (52 days old) were assigned to 4 groups, 10 per sex, and exposed to primary amyl acetate at target concentrations of 0, 300, 600, and 1200 ppm for 9 days, 6 hours per day. Rats were housed two per cage in stainless steel wire-mesh cages. All animals were housed separately by test group and sex. During exposures, animals were housed two per cage, separated by test group and sex in stainless steel wire-mesh cages; food and water was withheld during exposure, but available *ad libitum* at all other times. Animals were exposed in 1330 L stainless steel and glass inhalation chambers for 6 hours per day. Metering of the liquid primary amyl acetate into a heat glass evaporator generated vapours of the test material. Temperature and relative humidity within the inhalation chambers were monitored at least 4 times per exposure interval. Chamber concentrations were analysed approximately once per hour using a gas chromatograph equipped with a flame ionisation detector. Average chamber concentrations were within 2 to 5% of target concentrations. Animals were observed prior to, during, and following each exposure for signs of toxicity. Ophthalmic examinations were performed prior to first exposure, and at sacrifice. Body weights of all animals were recorded prior to exposure, on exposure days 2, 5, 6, 7, and the day following the final exposure. Serum chemistry and haematological evaluations were performed on blood samples collected from all rats on the day of sacrifice.

After eight exposures for female rats, and nine exposures for male rats, animals were individually housed in round polycarbonate metabolism cages and food and water consumption was measured for approximately 14-16 hours. Urine was collected while rats were housed in metabolism cages, food and water was available *ad libitum* during collection. All rats were sacrificed on the day following the final exposure. Gross examinations were performed and the liver, kidneys, lungs and testes (males only) from all animals were weighed. The following tissues from each animal were saved and fixed in 10% neutral buffered formalin: kidneys, liver, lungs, nasal turbinates, ovaries, testes, spleen, larynx, and trachea; nasal turbinates were perfused with fixative, and lungs were gently inflated with fixative. Bone marrow impression smears were obtained from each animal. Histological evaluations were performed on the bone marrow, kidneys, liver, lungs, nasal turbinates, ovaries, testes, spleen, larynx, and trachea from animals in the 1200 ppm exposure and control groups.

Statistical analysis: Results of quantitative continuous variables (e.g. body weight change) were intercompared for the 3 dosage groups and 1 control group by use of Bartlett's test for homogeneity of variance (Sokal and Rohlf, 1969), analysis of variance (ANOVA, Sokal and Rohlf, 1969), and Duncan's multiple range tests (Snedecor and Cochran, 1967). The latter was used, if the F for the ANOVA was significant, to delineate which groups differ from the control. If Bartlett's test indicates heterogeneous variances, all groups were

compared by an ANOVA for unequal variances (Brown and Forsythe, 1974a) followed, if necessary, by t-tests. For continuous data other than body weight change, organ weights, and food and water consumption, the statistical procedures were the same except Levene's test (Brown and Forsythe, 1974b) for equal variances were used instead of Bartlett's test and that t-tests or other appropriate tests were used instead of Duncan's multiple range test. The fiducial limit of 0.05 (two-tailed) was used as the critical level of significance for all comparisons.

Sokal, R.R. and Rohlf, F.J. 1969. Biometry, San Francisco, CA: W.H. Freeman and Company.

Snedecor, G.W. and Cochran, W.G. 1967. Statistical Methods. Ames, IO: Iowa State University Press.

Brown, M.B. and Forsythe, A.B. 1974a. The small sample behavior of some statistics which test the equality of several means. Technometrics 16: 129-132.

Brown, M.B. and Forsythe, A.B. 1974b. J. Amer. Statist. Assoc. 69: 364-367.

Year: 1984

GLP: no

Test substance: primary amyl acetate, purity 98.8%

Remark : Animals were observed daily for signs of toxicity prior to exposure, during exposure, and following exposure. The eyes of male and female rats were partially closed during exposure, however, when animals were removed from the chamber the animals appeared normal. The cause of the partially closed eyes is unknown, since no signs of eye irritation were present; no lesions were observed upon ophthalmologic examination of each animal at the end of the study. No other signs of toxicity were noted.

Results: Average chamber concentrations were within 2 to 5% of the target concentrations of 300, 600, and 1200 ppm. The test substance had a purity of 98.8 at initiation of the test, and a purity of 98.7% at termination.

There were no exposure-related clinical observations other than the eyes of the animals were partially closed during exposure. There were no treatment related effects observed with regard to body weight gain, food or water consumption, clinical chemistry, urinalysis, or hematological indices. No lesions were observed upon ophthalmologic examination of each animal at the end of the study. No treatment related effects were noted upon microscopic examination of the lungs, liver, kidneys, or testes (males only). There was a slight increase in absolute kidney weight and in relative kidney weight in males exposed to 1200 ppm, however, no treatment related tissue injury was identified in the kidneys.

Reliability: score = 1, valid with restriction; comparable to guideline study

Reference: Snellings, W.M. 1984. Union Carbide Corporation (unpublished report). Primary Amyl Acetate Acute and Nine-Day Inhalation Vapor Studies on Rats, Bushy Run Research Center, Project Report 47-103, dated October 16, 1984.

## 5. TOXICITY

ID: 628-63-7,624-41-9

DATE: 01.07.2006

(d)	Test substance:	Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate
	Species:	rat
	Strain:	CFN albino
	Sex:	male and female
	Route of Admin:	ingestion
	Exposure Period:	90 days (males), 91 days (females)
	Freq. of Treatment:	daily, 7 days/week
	Post Exposure Observation Period:	none
	Doses:	0.0, 0.1, 0.5, or 1.0% primary amyl acetate in diet, equivalent to: in males: 0, 68, 320, or 650 mg/kg/day in females: 0, 74, 350, or 720 mg/kg/day
	Control Group:	yes
	NOAEL:	1.0% amyl acetate in diet equivalent to: 650 mg/kg/day (males) 720 mg/kg/day (females)
	LOAEL:	NA
	Method:	Groups consisting of 20 CFN rats (45 days old), 10 males and 10 females, were fed a diet containing 0.1, 0.5, or 1.0% primary amyl acetate for 90 days (91 days for females). Rats were housed together in wire-bottom cages, 5 rats per cage. The quantity of diet consumed by each cage of rats was recorded, and the actual amount of primary amyl acetate consumed was determined.
		Diet was provided to each cage of rats in a glass container. The container was weighed when full; when nearly empty, it was weighed, refilled, and re-weighed. Rats were weighed prior to the start of the study, and weekly thereafter. After three months the rats were sacrificed. The livers and kidneys of each rat was dissected and weighed. All organs were grossly examined for signs of pathological changes or infection. The urinary bladder was examined for concretions. Tissue samples were taken for histological examination from the lung, kidney, liver, and urinary bladder from each of the dosed and control animals. Pancreas, stomach, duodenum and colon tissues were taken from a few representative rats at each dosage level. Differences in measured parameters were evaluated using the Rao method of analysis of variance (ANOVA). The fiducial limit of 0.05 (two tailed) was used to determine statistical significance.
	Year:	1958
	GLP:	no
	Test substance:	Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate, purity not specified
	Remark:	Dosages of primary amyl acetate in males were 650 mg/kg, and 720 mg/kg in females, based on amount of diet consumed per cage.
	Results:	One rat from the control, 0.1%, and 0.5% group died during the study. Gross and microscopic examination of tissues indicated that death in each case was caused by acute bronchopneumonia. No deaths



occurred in the highest dose group which received 1% primary amyl acetate in the diet.

Primary amyl acetate fed in the diet of rats for 90 days had no adverse effect, even at the highest concentration fed in the diet, 1% primary amyl acetate (650 mg/kg/day in males, 720 mg/kg/day in females). There were no significant differences observed in mortality, body weight gain, food consumption, liver weight, or histopathology for treated groups as compared to controls.

Rats in the 0.1% and 0.5% exposure groups displayed greater body weight gains than controls, however the effect was significant only among the 0.1% females. A minor depression in mean kidney weight was observed in the 0.5 and 0.1% groups, but this was considered an irrelevant artefact since it was not present in the 1.0% group and no pathological changes were observed upon histological examination of the kidney.

Reliability: score = 2, valid with restrictions; purity of test substance not specified, evaluation of haematology, urinalysis, and clinical chemistry parameters not performed.

Reference: Weil, C.S. 1958. Union Carbide Corporation (unpublished report). Results of 90-Day Feeding of Primary Amyl Acetates (mixed isomers), Mellon Institute of Industrial Research, University of Pittsburgh, Special Report 21-36, dated May 12, 1958.

## 5.5 GENETIC TOXICITY IN VITRO

### A. BACTERIAL IN VITRO TEST

#### (a) Critical study and preferred value

Test substance: primary amyl acetate (mixture of 3 isomers):

60% 1-pentyl acetate  
35% 2-methyl butyl acetate  
5% 3-methyl butyl acetate

Type: Salmonella/microsome bacterial mutagenicity assay (Ames test)

System of Testing: Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538;

Concentration: 0.01, 0.03, 0.1, 0.3, or 1.0 ug/plate

Metabolic Activation: with and without

Method other: according to: Ames, B.N., McCann, J. and Yamasaki, F. 1975. Methods for detecting carcinogens and mutagens with Salmonella/mammalian-microsome mutagenicity test. Mutation Research 31: 347-364.

Primary amyl acetate was dissolved in absolute ethanol; all dilutions were made in ethanol on the day of testing. A preliminary toxicity test was performed to determine the level of cytotoxicity of the test material to the tester strains. Ten doses, from 0.001 to 50 ul per plate were tested. The top four doses, 50, 10, 3 and 1 ul/plate exhibited cytotoxicity. Based on these results, mutagenicity testing was

performed with 5 doses of 1.0, 0.3, 0.1, 0.03 and 0.01 ul/plate. All doses were tested in triplicate, in the presence and absence of metabolic activation, in 5 tester strains. Arochlor 1254-induced rat liver homogenate (S9) was prepared fresh on the day of testing from male Sprague Dawley rats. For tests with metabolic activation, 0.5 ml of S9 mix containing 50 ul S9 was added per plate. Solvent and positive controls were run concurrently with the test material. For all tests, the solvent control was 100% ethanol (50 ul/plate). The positive control used for all tester strains in tests with metabolic activation was 2-aminoanthracene (10 ug/plate). The positive controls used for each tester strain in tests without metabolic activation were as follows:

TA98, TA1538: 4-nitro-o-phenylenediamine (10 ug/plate)  
TA100, TA1535: sodium azide (10 ug/plate)  
TA 1537: 9-aminoacridine (60 ug/plate).

All test and control concentrations were run in triplicate plates, and the mean plate count and standard deviation was determined. An Artek Model 880 Colony Counter was used to count bacterial colonies. Data were analyzed according to the method of deSerres and Shelby (1979). The fiducial limit of 0.05 was used to determine statistical significance.

DeSerres, F.D. and Shelby, F.D. 1979. Recommendations on data production and analysis using the Salmonella /microsome mutagenicity assay. Mutation Research 64: 159-165.

A compound is considered a bacterial mutagen if the number of revertant colonies is at least twice the solvent control for at least one dose level and there is evidence of a dose-related increase in the number of revertant colonies. If there is no evidence of a dose-related increase in the number of revertant colonies, and the number of revertant colonies is not twice the solvent control, the test substance is not considered a bacterial mutagen. If a test produces a marginal or weak result, the test is repeated.

Year: 1983

GLP: no

Test substance: primary amyl acetate (mixture of 3 isomers):  
60% 1-pentyl acetate  
35% 2-methyl butyl acetate  
5% 3-methyl butyl acetate

Result: With metabolic activation: negative  
Without metabolic activation: negative

No evidence of mutagenicity was observed at any of the tested doses, either by evidence of a dose-response or by a doubling of the number of revertant colonies relative to control values. In tests with and without metabolic activation, toxicity was observed at 1 ul/plate with all strains as a slight reduction in the number of colonies per plate. All strains exhibited a positive mutagenic response with positive controls tested both with and without metabolic activation. Negative (ethanol)

control spontaneous revertant rates were within the historical range of the laboratory.

Remark: Primary amyl acetate did not produce a mutagenic response in any of the Salmonella tester strains, in the presence or absence of metabolic activation.

Reliability: score = 2, valid with restriction, comparable to guideline study, however purity of test substance not confirmed

Reference: Hengler, W.C. and Slesinski, R.S. 1983. Union Carbide Corporation (unpublished report). Primary Amyl Acetate, Salmonella/ Microsome (Ames) Bacterial Mutagenicity Assay. Bushy Run Research Center Project Report 46-12, February 16, 1983.

## B. NON-BACTERIAL IN VITRO TEST

### (a) Critical study and preferred value

Type: *In vitro* chromosomal aberration assay

System of Testing: Rat lymphocytes

Concentration: 0, 20.5, 40.9, 81.9, 163.8, 327.5, 655, and 1310 µg/ml (4 hour treatment, with and without S9)  
0, 10.2, 20.5, 40.9, 81.9, 163.8, 327.5, 655, and 1310 µg/ml (24 hour treatment, without S9)

Metabolic Activation: with and without

Result: With metabolic activation: negative

Without metabolic activation: negative

There was no increase in the incidence of chromosomal aberrations in rat lymphocyte cultures treated with Primary Amyl Acetate (mixed isomers) for 4 or 24 hours in the absence of metabolic activation or 4 hours in the presence of metabolic activation. Cultures treated with positive controls displayed significantly higher incidences of chromosomal aberrations in all assays. Solvent control spontaneous aberration rates were within the historical range of the laboratory.

Method: Study conducted to comply with the following guidelines:  
OECD #473, *In vitro* mammalian chromosome aberration test;  
USEPA Health effects testing guidelines, OPPTS 870.5375, *In vitro* mammalian chromosome aberration test;  
EC B.10 Mutagenicity - *In vitro* mammalian chromosome aberration test.

Primary Amyl Acetate (mixed isomers) was tested in rat lymphocyte cultures at 20.5, 40.9, 81.9, 163.8, 327.5, 655, and 1310 µg/ml both in the presence and absence of metabolic activation for 4 hours and then harvested 20 hours after termination of exposure. Cultures were also treated continuously for 24 hours in the absence of S9 with the above concentrations plus an additional lower concentration of 10.2 µg/ml and harvested immediately after the termination of the exposure. The test material was first dissolved in dimethyl sulfoxide (DMSO) and further diluted (1:100) with culture medium to obtain desired concentrations. Solvent control cultures were exposed to 1% DMSO. Positive control cultures were exposed to mitomycin C (MMC)

without S-9 and cyclophosphamide (CP) with S-9. All stock solutions were analysed by gas chromatography with flame ionization detection (GC/FID) to verify concentrations.

Blood samples were collected by cardiac puncture from 10 week old male CD ISG (Outbred Crl:CD (SD)IGSBR) rats, following euthanasia with carbon dioxide. In the assay, blood samples from individual rats were pooled and whole blood cultures were set up in RPMI 1640 medium (with 25 mM HEPES, GIBCO, Grand Island, New York) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO), antibiotics and antimycotics (Fungizone 0.25 µg/ml; penicillin G, 100 u/ml; and streptomycin sulfate, 0.1 mg/ml; GIBCO), 30 µg/ml PHA (HA16, Murex Diagnostics Ltd., Dartford, England), and an additional 2 mM L-glutamine (GIBCO). Cultures were initiated by inoculating approximately 0.5 ml of whole blood/5 ml of culture medium. Cultures were set up in duplicate at each dose level in T-25 plastic tissue culture flasks and incubated at 37°C.

Short Treatment without Metabolic Activation:

Forty-eight hours ( $\pm$  1 hour) after initiation of the cultures, the cell suspension was dispensed into 15 ml sterile disposable centrifuge tubes (5.5 ml/tube, two cultures per dose level). The cells were sedimented by centrifugation and the culture medium removed and saved. The cells were exposed to medium (RPMI 1640, HEPES, and antibiotics) containing the test or positive or solvent control treatments for 4 hours ( $\pm$  30 minutes) at 37°C and the exposure was terminated by washing the cells with culture medium. The cells were then placed in individual sterile disposable tissue culture flasks (T-25) along with approximately 4.5 ml of the original culture medium until the time of harvest. The cultures were harvested 24 hours ( $\pm$  1 hour) after treatment initiation (*i.e.*, 20 hours,  $\pm$  1 hour after treatment termination).

Continuous Treatment without Metabolic Activation:

Cultures were treated continuously with the test material for 1.5 times the normal cell cycle length. The solvent control, positive control, and test material were added directly to the culture flasks 48 hours ( $\pm$  1 hour) after initiation of the cultures, and the cultures were harvested 24 hours ( $\pm$  1 hour) later.

Treatment Procedure using Metabolic Activation (S9):

Only short treatment (4 hours  $\pm$  30 minutes) procedure was used with S9. Forty-eight hours ( $\pm$  1 hour) after initiation of the cultures, the cell suspension was dispensed into 15 ml sterile disposable centrifuge tubes (5.5 ml/tube, two cultures per dose level). The cells were sedimented by centrifugation and the culture medium removed and saved. The cells were exposed to medium (RPMI 1640, HEPES, antibiotics, and the S9 mix) containing the test or positive or solvent control treatments for 4 hours ( $\pm$  30 minutes) at 37°C and the

exposure was terminated by washing the cells with culture medium. The cells were then placed in individual sterile disposable tissue culture flasks (T-25) along with 4.5 ml of the original culture medium until the time of harvest. The cultures were harvested at 24 hours ( $\pm 1$  hour) after treatment initiation (*i.e.*, 20 hours,  $\pm 1$  hour after treatment termination).

Colcemid (1  $\mu\text{g}/\text{culture}$ ) was added 2-3 hours prior to harvest. The cells were swollen by hypotonic treatment (0.075 M KCl), fixed with methanol:acetic acid (3:1), dropped on microscope slides, and stained in Giemsa. Mitotic indices were determined as the number of cells in metaphase among 1000 cells/replicate and expressed as percentages. Slide scoring for mitotic indices and chromosomal aberrations were performed at the testing facility. Initially, slides from the short treatment (with and without S9) were evaluated for cytogenetic analysis. Slides from the continuous treatment without S9 were evaluated only when the results with short treatment yielded negative findings. Slides from the solvent controls, positive controls, and at least three concentrations of the test material were selected for cytogenetic analysis.

One hundred metaphases/replicate were examined from coded slides for structural abnormalities. The number of cells examined for structural abnormalities was reduced to 50 metaphases per replicate if high numbers of aberrations were observed (approximately 20%). The microscopic coordinates of metaphases containing aberrations were recorded. Only those metaphases that contained  $42 \pm 2$  centromeres were scored with the exception of cells with multiple aberrations, in which case accurate counts of the centromeres was not possible. Structural chromosomal abnormalities counted include chromatid and chromosome gaps, chromatid breaks and exchanges, chromosome breaks and exchanges, and miscellaneous (chromosomal disintegration, chromosomal pulverization, etc.). Those cells having five or more aberrations/cell were classified as a cell with multiple aberrations. Chromatid gaps and chromosome gaps were not included in calculations of total cytogenetic aberrations. In addition, a total of 100 metaphases/replicate were examined for incidence of polyploidy.

**Evaluation Criteria:** For a test to be acceptable, the chromosomal aberration frequency in the positive control cultures must be significantly higher than the solvent controls. The aberration frequency in the solvent control should be within reasonable limits of the laboratory historical values. A test chemical is considered positive in this assay if it induces a significant dose-related and reproducible increase in the frequency of cells with aberrations.

**Statistical Analysis:** The proportions of cells with aberrations (excluding gaps) were compared by the following statistical methods. At each dose level, data from the replicates were pooled. A two-way contingency table

was constructed to analyze the frequencies of aberrant cells. An overall Chi-square statistic, based on the table, was partitioned into components of interest. Specifically, statistics were generated to test the global hypotheses: 1) no difference in the average number of cells with aberrations among the dose groups, and 2) no linear trend of increasing number of cells with aberrations with increasing dose. An ordinal metric (0, 1, 2, etc.) was used for the doses in the statistical evaluation. If either statistic was found to be significant at  $\alpha = 0.05$ , versus a one-sided increasing alternative, pairwise tests (*i.e.* control vs. treatment) were performed at each dose level and evaluated at  $\alpha = 0.05$ , again versus a one-sided alternative. Polyploid cells were analyzed by the Fisher Exact probability test. The numbers of polyploid cells were pooled across replicates for the analysis and evaluated at  $\alpha = 0.05$ . The data was analyzed separately based on the presence or absence of S-9 and based on the exposure time.

Year: 2006  
GLP: yes  
Test substance: Primary Amyl Acetate(mixed isomers), purity 99.7%  
Remark: Primary Amyl Acetate did not induce a significant increase in the incidence of cells with chromosomal abnormalities at any of the concentrations evaluated.

Results: Approximately 48 hours after initiation of whole blood cultures, rat lymphocytes were treated for 4 (absence and presence of S9) and 24 hours (absence of S9) with primary amyl acetate-mixed isomers at target concentrations up to 1310  $\mu\text{g/ml}$  (10 mM). There was minimal toxicity in cultures exposed to primary amyl acetate-mixed isomers for 4 hours, with relative mitotic indices ranging from 72.6 to 82.2% in the absence of S9 and 76.7 to 103.9% in the presence of S9. In cultures treated continuously for 24 hours in the absence of S9 activation, the highest concentration was severely toxic as evident by no mitotic figures. The remaining concentrations had relative mitotic indices ranging from 47.0 to 104.6% compared to the solvent control values.

In the 4 hour non-activation assay, the frequency of cells with aberrations in the solvent control was 1.0% and the corresponding values at treatment levels of 327.5, 655, and 1310  $\mu\text{g/ml}$  were 1.0, 1.5, and 1.5%, respectively. In the activation assay, cultures treated with the test material at concentrations of 327.5, 655, and 1310  $\mu\text{g/ml}$  had aberrant cell frequencies of 1.5, 2.0, and 1.5%, respectively as compared to the solvent control value of 1.0%. Statistical analyses of these data did not identify significant differences between the solvent control and any of the treated cultures without or with S9 activation. The frequencies of aberrant cells observed in the test material treated cultures were within the laboratory historical background range.

In the non-activation assay, where cultures were treated continuously for 24 hour with the test material, the frequencies of aberrant cells in the solvent control was 1.0% and the corresponding values at concentration levels of 163.8, 327.5, and 655 µg/ml were 0.0, 1.0, and 1.5%, respectively. There were no statistically significant differences between the test material treated cultures and the solvent control values and all values were within the laboratory historical background range.

Significant increases in the frequency of cells with aberrations were observed in cultures treated with the positive control chemicals. Aberrant cell frequencies in MMC (without S9, 4 hour treatment), CP (with S9, 4 hour treatment) and MMC (without S9, 24 hour treatment) cultures were 35.0%, 14.0%, and 27.0%, respectively.

It was concluded that under the experimental conditions used, Primary Amyl Acetate was non-genotoxic in this *in vitro* chromosomal aberration test.

Reliability:  
Reference:

score = 1, valid without restriction; GLP guideline study  
Charles, G.D., Schisler, M.R., and Kleinert, K.M. 2006. Evaluation of Primary Amyl Acetate (mixed isomers) in an *in vitro* chromosomal aberration assay utilizing rat lymphocytes. Laboratory Project Study ID 051192. The Dow Chemical Company, Midland, MI.

- (b) Test substance: primary amyl acetate (mixture of 3 isomers):  
60% 1-pentyl acetate  
35% 2-methyl butyl acetate  
5% 3-methyl butyl acetate
- Type: mutagenicity assay
- System of Testing: Chinese Hamster Ovary (CHO) Mutation test
- Concentration: 0.02, 0.04, 0.06, 0.08, 0.10% (v/v) without metabolic (S9) activation  
0.04, 0.06, 0.08, 0.10, 0.20, 0.25, 0.30% (v/v) with S9 activation
- Metabolic Activation: with and without
- Method other: according to: O'Neill J.P. et al. 1977. A quantitative assay of mutation induction at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary cells (CHO/HGPRT System): Development and definition of the system. Mutation Research 45: 91-101. and O'Neill, J.P. and Hsie, A.W. 1979. Phenotypic expression time of mutagen-induced 6-thioguanine resistance in Chinese hamster ovary cells (CHO/HGPRT system). Mutation Research 59: 109-118.

Primary amyl acetate was dissolved in absolute ethanol to a concentration of 20 µl/ml; all dilutions were made in ethanol on the day of testing. A preliminary toxicity test was performed in the presence and absence of S9 metabolic activation to determine the level of cytotoxicity (growth inhibition) of the test material. CHO cells in culture were treated for 5 hours with primary amyl acetate at concentrations of 1.0, 0.5, 0.3, 0.1, 0.6, 0.3 and 0.1%. The top two doses, 1% and 0.5% exhibited cytotoxicity (over 90% of cells killed). Based on these results, CHO cell cultures were exposed for 5 hours to

a minimum of five concentrations between 0.02% to 0.4% . All doses were tested in duplicate cultures in the presence and absence of metabolic activation.

CHO cells (CHO-K1-BH, subclone D1) were obtained from Abraham Hsie (Oak Ridge National Laboratory). Arochlor 1254-induced male rat liver homogenate (S9) was purchased commercially from Meloy Laboratories, Springfield, VA. The S-9 metabolic activation system was prepared fresh on the day of testing. Concurrent solvent and positive controls were run concurrently with the test material. For test cultures exposed in the presence of metabolic activation, a volume of 1 ml of the S9 activation system was added per 4 ml of culture medium.

Cells were incubated for 5 hours with appropriate concentrations of the test material or vehicle control at 37 °C. The test medium is then removed, the cells rinsed, and re-fed with fresh medium. Cytotoxicity was assessed 20-24 hr after treatment. After a 2-3 day interval, cells are re-cultured, and allowed to replicate for a 7-day expression interval. Cells are then re-plated into 5 plates at a concentration of  $2 \times 10^5$  cells per plate and fed selective medium containing 6-thioguanine (TG); plating efficiency is evaluated in 4 additional plates with cells fed medium without TG. All cultures are incubated for an additional 6-8 days to allow cells to replicate. The cell medium was then discarded and the cell colonies fixed and stained for counting. The number of colonies in selection and plating efficiency cultures are electronically counted, checked by manual counts, and the data recorded as mutants per  $10^6$  total cells, and mutants per  $10^6$  viable cells. Solvent (ethanol, 20 ul/ml) and positive control (ethylmethanesulfonate (EMS), 200 ug/ml) were run concurrently with the test material.

Statistical analysis: Data from the CHO test were analysed after transformation of the mutation frequencies (MF) according to the conversion method of Box and Cox (1964). The corrected mutation frequency for each plate is increase by 1.0 (to eliminate zeros), and raised to the 0.15 power. If the mutation frequency for both solvent controls is zero, the historical variance for negative control data is used for statistical comparison. Parametric analysis of mutation data by the Student's t-test is performed with the transformed data. Positive controls for the CHO mutation test were run concurrently to assess the sensitivity of the assays in comparison to historical controls. Data for positive control agents were at least 10 times the concurrent negative control value. The degree of significance ( $p < 0.05$ ) for mutation values is indicative of a statistical difference from concurrent solvent controls.

Box, G.E.P. and Cox, D.R. 1964. An analysis of transformation. J. Royal Statistical Society 26: 211-252.

Year: 1983  
GLP: no



Test substance:	primary amyl acetate (mixture of 3 isomers): 60% 1-pentyl acetate 35% 2-methyl butyl acetate 5% 3-methyl butyl acetate
Result:	With metabolic (S9) activation: negative Without metabolic activation: negative Cytotoxicity (less than 10% survival to total lethality) was observed in cell cultures exposed to 0.3, 0.4 and 0.5% primary amyl acetate. Cell viability in cultures with S9 activation was acceptable at and below the concentration of 0.25% primary amyl acetate. Cell viability in cultures without metabolic activation was acceptable at and below the concentration of 0.10% primary amyl acetate. In cultures with acceptable levels of cytotoxicity, primary amyl acetate did not produce any dose-related or statistically significant increases in the frequency of mutations of CHO cells, both in the presence and absence of S9 metabolic activation. The solvent control did not induce an increase in mutation frequency. The positive control induced a significant increase in the incidence of mutant colonies as compared to solvent controls.
Remark:	Primary amyl acetate did not produce a mutagenic response in CHO cells, in the presence or absence of metabolic activation.
Reliability:	score = 2, valid with restriction, comparable to guideline study, however purity of test substance not confirmed
Reference:	Slesinski, R.S. 1983. Union Carbide Corporation (unpublished report). Primary Amyl Acetate, <i>In vitro</i> Mutagenesis Studies. Bushy Run Research Center, Project Report 46-109, November 14, 1983.
(b) Test substance:	primary amyl acetate (mixture of 3 isomers): 60% 1-pentyl acetate 35% 2-methyl butyl acetate 5% 3-methyl butyl acetate
Type other:	cytogenetic assay
System of Testing:	Chinese Hamster Ovary (CHO) Sister Chromatid Exchange (SCE) test
Concentration:	0.03 - 0.3% with metabolic activation 0.04 - 0.15% without metabolic (S9) activation
Metabolic Activation:	with and without
Result:	with metabolic (S9) activation: negative without metabolic activation: negative
Method other:	Primary amyl acetate was dissolved in absolute ethanol to a concentration of 20 ul/ml; all dilutions were made on the day of testing. Based upon cytotoxicity testing the CHO HGPRT Mutation test [see Section 5.5 B(a)], 5 doses, with concentration ranges of 0.03% to 0.30% (v/v) were tested with S9 metabolic activation, a concentration range of 0.04 to 0.15% (v/v) was selected for cultures in the absence of metabolic activation. Test concentrations were chosen which are moderately toxic (40-50% survival) which allow sufficient numbers of cells in the second division (M2) for determination of SCEs. The incidence of SCEs was determined in the highest three doses which did not produce excessive toxicity.

CHO cells (CHO-K1-BH, subclone D1) were obtained from Abraham Hsie (Oak Ridge National Laboratory) and subcultured in Ham's F12 Medium supplemented with 10% heat-inactivated fetal bovine serum and lacking in hypoxanthine and thymidine. Arochlor 1254-induced male rat liver homogenate (S9) was purchased commercially from Litton Bionetics, Kensington, MD. The S-9 metabolic activation system was prepared fresh on the day of testing. A volume of 1 ml S9 mix was added per 4 ml of culture medium. Concurrent solvent (ethanol, 5 ul/ml) and positive controls (ethylmethanesulfonate (EMS), 100 ug/ml) were run concurrently with the test material.

Appropriate concentrations of the test substance or solvent control were added to cell cultures and 3 ul/ml BUdR was added. Cultures that did not receive S9 metabolic activation were treated with the test agent and BUdR for five hours; the media was then removed, the cells rinsed, and fresh medium containing BUdR was added. Treated cells were incubated for at least 24 hours to allow two rounds of cell division. When the S9 metabolic activation system was added, cultures were treated with the test agent and BUdR for 2 hours, then rinsed and incubated for 30 to 40 hours.

Colcehicine (2 ug/ml) was added to culture flasks 1-2 hr prior to harvesting to arrest cells in mitosis. Cells were then harvested and prepared for SCE staining. Chromosome spreads were stained with Hoechst dye 33258 for 20 minutes, rinsed, then immersed in Sorenson's buffer and exposed to a high intensity sunlamp for 15-30 minutes. Slides were coded and scored blind; slides were decoded only after scoring of all slides had been completed. A total of 25 cells per concentration was studied for SCE production using duplicate cultures. SCE production was determined for the highest three doses which did not produce excessive cytotoxicity. The number of SCEs/cell, mean number of SCEs/chromosome and the level of statistical significance of the increases above the concurrent solvent control values were calculated and recorded.

Statistical analysis: Statistical methods: Analysis of mutation frequencies in the CHO Sister Chromatid Exchange (SCE) assay follows the procedure which employs the Box-Cox Transformation to transform data before parametric analyses. The corrected mutation frequency for each plate is increase by 1.0 (to eliminate zeros), and raised to the 0.15 power. If the mutation frequency for both solvent controls is zero, the historical variance for negative control data is used for statistical comparison. Data was analysed by Duncan's multiple range test by comparing individual test groups wit the combined negative control groups. The degree of significance ( $p < 0.05$ ) for SCE incidences is indicative of a statistical difference from concurrent solvent controls.

Box, G.E.P. and Cox, D.R. 1964. An analysis of transformation. J. Royal Statistical Society 26: 211-252.

	A material is considered positive when there is a doubling of the SCE frequency at any single concentration relative to concurrent controls, or there is a statistically significant ( $p < 0.05$ ) increase in the incidence of SCE relative to concurrent controls at two or more consecutive concentrations. When a dose response relationship is not evident, testing may be repeated to clarify the results.
Year:	1983
GLP:	no
Test substance:	primary amyl acetate, (mixture of 3 isomers): 60% 1-pentyl acetate 35% 2-methyl butyl acetate 5% 3-methyl butyl acetate
Results:	No significant increase in the SCE frequency was observed with any of the cultures treated with primary amyl acetate. Primary amyl acetate did not produce a dose-related increase in the frequency of sister chromatid exchanges (SCEs) in CHO cells with and without an S9 metabolic activation system.
Reliability:	score = 2, valid with restriction, comparable to guideline study, however purity of test substance not confirmed
Reference:	Slesinski, R.S. 1983. Union Carbide Corporation (unpublished report). Primary Amyl Acetate, <i>In vitro</i> Mutagenesis Studies. Bushy Run Research Center, Project Report 46-109, November 14, 1983.

## 5.6 GENETIC TOXICITY IN VIVO

### (a) Critical study and preferred value

Test substance:	1-pentyl acetate (Mallinckrodt)
Remark:	1-pentyl acetate is the major component of Primary Amyl Acetate, and is present in the mixture at a concentration of 65%
Test species/strain:	<i>Melanoplus sanguinipes</i> (grasshopper)
Test method:	Grasshopper embryo system cytogenetic assay The grasshopper embryo system can be used to detect agents that produce mitotic arrest and anaphase abnormalities. The grasshopper is a useful organism for cytological studies because of its large chromosomes and relatively low diploid number. In grasshopper embryos, cell generation time is very short and normal cell divisions can be maintained for up to 5 cell cycles. In this study, grasshopper embryos (intact chorions) were obtained from eggs produced by a non-diapause laboratory strain of <i>Melanoplus sanguinipes</i> . After collection, egg pods were transferred to moistened vermiculite in capped Styrofoam cups. Embryos used in this study were at embryonic development day 7-9. Three randomly selected embryos were suspended in Coplin jars (95 ml volume); 0.1, 0.2 or 0.5 ml amyl acetate was placed in the bottom of each jar. The screw top was then tightly sealed with petroleum jelly. Embryos exposed to air only and distilled water served as untreated controls. Jars were maintained at 24 degrees C for 8 or 16 hours. Volumes of less than 0.5 ml of the test material vaporized completely within the jars. Immediately after treatment, squash slide preparations of embryos were prepared and fixed. After fixation, embryos were stained. A minimum of 3000 cells

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	per embryo were scored to determine mitotic index (MI). The anaphase to metaphase (A/M) ratio was determined by counting the number of anaphases present for every 100 metaphases.
GLP:	no
Test results:	negative
Lowest dose producing toxicity:	N/A
Effect on Mitotic Index or P/N Ratio:	none
Genotoxic effects:	none
Remark:	1-Pentyl acetate did not display mitosis-arresting effects in any of the treated embryos. There was no significant differences in mitotic index and metaphase ratio between control (water and air) and amyl acetate treated embryos.
Reliability:	score = 3, invalid; unsuitable test system
Reference:	Liang, J.C., Hsu, T.C. and Henry, J.E. 1983. Cytogenetic assays for mitotic poisons. The grasshopper embryo system for volatile liquids. Mutation Research 113: 467-479.

**5.7 CARCINOGENICITY**

No data available

**5.8 TOXICITY TO REPRODUCTION****(a) Critical study and preferred value**

Test substance:	Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate
Species:	rat
Strain:	Fischer 344
Sex:	male and female
Route of Admin:	inhalation
Exposure Period:	14 weeks
Freq. of Treatment:	6 h/day, 5 days/week
Post Exposure	
Observation Period:	one month
Doses:	0, 100, 300, 500 ppm primary amyl acetate
Control Group:	yes
NOAEL:	500 ppm
LOAEL:	
Method:	Equivalent to OECD 413 Male and female 344 rats (55 days old at test initiation) were assigned to 4 groups, 20 per sex per group, and exposed to primary amyl acetate at target concentrations of 0, 100, 300, and 500 ppm for 6 hours per day, 5 days per week, for 14 weeks. Animals were observed prior to, during, and after each exposure; on non-exposure days, animals were observed daily. Recovery animals were observed daily. After the final exposure, half the exposed animals (10 rats per sex per exposure group) were sacrificed; the remaining animals (recovery group, 10 rats per sex per exposure group) were held for a one-month observation period and then sacrificed.

All rats were housed separately by sex and test group. During exposures, animals were housed two per cage, separated by sex and test group in stainless steel wire-mesh cages. Cage positions were rotated weekly in a predetermined pattern within each chamber to compensate for any possible but undetected variation in chamber conditions. Food and water was withheld during exposure but available *ad libitum* at all other times. Animals were exposed in 1330 L stainless steel and glass inhalation chambers for 6 hours per day.

Metering of the liquid primary amyl acetate into a heated glass evaporator generated vapours of the test material. The resultant vapor was carried into the chamber by a countercurrent air stream that entered at the bottom of the evaporator. Chamber concentrations were analysed approximately once per hour using a Perkin-Elmer Model 3920B gas chromatograph equipped with a flame ionisation detector. The test material was determined to have a purity of 98.97% at test initiation, and a purity of 98.7% at termination. Average chamber concentrations were within 1 to 3% of the target concentrations.

All animals were acclimated to the exposure chambers (exposed to air only) for two days prior to initiation to the test article exposure regimen. Animals were observed prior to, during, and following each exposure for signs of toxicity. Ophthalmic examinations were conducted prior to first exposure, and at sacrifice. Body weights of all animals were recorded prior to exposure, weekly during exposure, and the day following the final exposure. Body weights for animals held during the post-exposure recovery period were recorded weekly and just prior to sacrifice. Serum chemistry and haematological evaluations were performed on blood samples collected from all rats on the day of sacrifice. Ten rats per sex per exposure group were individually housed in round polycarbonate metabolism cages; food and water consumption was measured for approximately 14-16 hours following 68 (females) or 67 (males) exposures. Urine was collected while rats were housed in metabolism cages, food and water was available *ad libitum*. Food and water consumption was not measured for recovery rats.

Upon sacrifice, gross examinations were performed and selected tissues saved; the brain, liver, kidneys, spleen, lung, and testes (males only) from all animals were weighed at sacrifice. Histological evaluation was performed on selected tissues (male and female gonads organs, spleen, lung, nasal turbinates, thymus, urinary bladder, adrenals, brain, parathyroids, heart, kidneys, larynx, thyroid, pituitary gland, gastrocnemius muscle, liver, sciatic nerve, and trachea) from animals in the high dose (500 ppm) exposure and control groups.

Statistics:

Results of quantitative continuous variables were intercompared among the three exposure groups and one control group by use of Bartlett's homogeneity of variance, analysis of variance (ANOVA),

	and Duncan's multiple range tests. The fiducial limit of 0.05 (two-tailed) was used as the critical level of significance for all comparisons.
Year:	1985
GLP:	yes
Test substance:	primary amyl acetate, purity 98.97%
Remark:	Control group males in this study displayed greater than normal body weight gains. The biological significance of the decreased weight gain is inconclusive, since the effect was not intensified with increasing concentration, and the weight gain of exposed animals was comparable to that of the historical controls for the laboratory. Testicular weights were not significantly different between groups when compared relative to body weight; however, the mean absolute weight of the testes was decreased in exposed males when compared to the larger control males.
Results:	<p>There were no signs of toxicity in any of the exposure groups. Among females, there were no exposure-related effects observed on body weight gain or absolute body weight during the study. The 100 ppm females had significantly higher body weight gains and/or absolute body weights at various intervals during the exposure period, but not during the recovery period. No lesions were observed upon macroscopic examination of an extensive list of tissues, including female ovaries, vagina, cervix, uterus and fallopian tubes. No pathology was observed upon histological examination of the ovaries.</p> <p>Among males, all exposure groups displayed similar, slight decreases in body weight gains. There was no statistical difference between control and exposed groups in body weight gains in animals allowed to recover for 4 weeks after the final exposure. The absolute weight of the testes in all male exposure groups were significantly decreased, however, there was no corresponding change in the relative weight of the testes in these groups. The decrease in absolute weight of the testes was unrelated to concentration and was not considered to be biologically significant; microscopic examination of the testes revealed no abnormalities. No lesions were observed upon macroscopic examination of an on an extensive list of tissues, including male testes, prostate, and associated gland and epididymis. There were no differences in organ weights in animals at the end of the 4-week post-exposure recovery period.</p>
Reliability:	score = 1, valid without restriction; comparable to guideline study
Reference:	Klönne, D.R. 1985. Union Carbide Corporation (unpublished report). Primary Amyl Acetate Acute Fourteen Week Inhalation Vapour Study on Rats. Bushy Run Research Center, Project Report 48-14, October 8, 1985.

## 5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

- (a) Critical study and preferred value  
Test substance: Primary Amyl Acetate, mixture of approximately 65%

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	1-pentyl acetate and 35% 2-methyl-1-butyl acetate
Species:	rabbit
Strain:	New Zealand White
Sex:	female
Route of Admin:	inhalation
Exposure Period	gestational days (gd) 6 through 18
Freq. of Treatment:	6 hours/day
Duration of Test	dams sacrificed on gd 29
Doses:	0, 500, 1000, or 1500 ppm
Control Group:	yes
NOAEL (Maternal Toxicity):	1000 ppm
NOAEL (Teratogenicity):	>1500 ppm
NOAEL (Develop. toxicity):	>1500 ppm
Method other:	<p>Seventy virgin female rabbits were mated to “proven” males from the Bushy Run Research Center breeding colony. On each mating day, successfully mated females were assigned to one of the three exposure groups or to the air-only control group. Each group containing 15 female mated New Zealand White rabbits. All does were observed twice daily for morbidity and mortality, and once per day for clinical signs of toxicity. In addition, during exposures, animals were observed from outside their respective exposure chambers for overt signs. Rabbits were exposed to primary amyl acetate vapor at concentrations of 500, 1000 or 1500 ppm for 6 hours/day on gestational days 6 through 18. Rabbits were housed individually in stainless steel wire-mesh cages. During exposures, animals were housed individually, separated by test group in stainless steel wire-mesh cages. Cage positions were rotated daily in a predetermined pattern within each chamber to compensate for any possible but undetected variation in chamber conditions. Food and water was withheld during exposure but available <i>ad libitum</i> at all other times. Animals were exposed in 4320 L stainless steel and glass inhalation chambers for 6 hours per day. Metering of the liquid primary amyl acetate into a heat glass evaporator generated vapours of the test material. Approximately nine samples were collected from each chamber during each exposure interval. Chamber concentrations were analysed by flame ionization gas chromatography and were within 2-5% of target concentrations. All maternal exposures were discontinued from gestation day 19 through study termination. Clinical observations were conducted daily, and body weights were measured on gestation day 0, 6, 12, 18, 24, and 29. Maternal food consumption was measured daily throughout the study (gd 0 through 29). All dams were sacrificed on gestational day 29. Ovarian corpora lutea of pregnancy were counted and the status of implantation sites (i.e. resorptions, dead and live fetuses) were identified and recorded. Live fetuses were euthanized by intraperitoneal injection of sodium pentobarbital. All live and dead fetuses were counted, weighed, and examined for external abnormalities including cleft palate. All live fetuses were examined for thoracic and abdominal visceral abnormalities. One half of the live fetuses were decapitated and their heads prepared for examination of craniofacial structures. All fetuses</p>

	were processed and examined for skeletal malformations and variations.
Data Analysis:	<p>The unit of comparison was the pregnant dam or the litter. The data for quantitative continuous variables were inter-compared for the exposure groups and the control group by use of Levene's test for equality of variances, analysis of variance (ANOVA), and t-tests. The t-tests were used when the F value from the ANOVA was significant. When Levene's test indicated similar variances, and the ANOVA was significant, a pooled t-test was used for pair-wise comparisons. When Levene's test indicated heterogeneous variances, all groups were compared by an ANOVA for unequal variances followed, when necessary, by a separate variance t-test for pair-wise comparisons.</p> <p>Non-parametric data obtained following laparohysterectomy were statistically evaluated using the Kruskal-Wallis test followed by the Mann-Whitney U test, when appropriate. Incidence data were compared using Fisher's Exact Test. With the exception of the data analysis for fetal malformations and variations, all statistical analyses were performed using BMDP Statistical software (Dixon, 1990). Incidence data for fetal malformations and variations were analyzed using program developed by Bushy Run Research Center. For all statistical tests, the probability value of <math>&lt; 0.05</math> (two-tailed) was used as the critical level of significance.</p> <p>Dixon, W.J. 1990. BMDP Statistical Software. University of California Press, Berkeley, CA.</p>
Year:	1994
GLP:	yes
Test substance:	primary amyl acetate, purity 99.1%, mixture composed of 1-pentyl acetate, 65.2% 2-methyl-1-butyl acetate, 33.9%
Remark:	Exposure of pregnant New Zealand White rabbits during organogenesis to primary amyl acetate vapour at 0, 500, 1000 or 1500 ppm resulted in maternal toxicity at 1500 ppm and no evidence of fetotoxicity or developmental toxicity.
Results:	<p>No mortality occurred during this study. The pregnancy rate was equivalent for all groups and ranged from 87 to 100%. Exposure-related clinical signs observed during the study included full food hoppers for several animals exposed to 1500 ppm.</p>
Maternal toxicity:	Maternal toxicity was observed in the 1500 ppm group as evidenced by decreases in gestational body weight were observed during the first six days of exposure (gd 6-12) and reduced food consumption during the entire exposure interval. Body weight gain was reduced in the 1500 ppm group for the entire exposure interval. There were no exposure-related necropsy findings in dams at scheduled sacrifice on gd 29. There were no statistically significant differences in terminal body weight. However, corrected body weights were decreased in the 1500 ppm group.
Pregnancy/litter data:	One female in the 500 ppm group aborted on gd24. Two females from the control and 1500 ppm groups were not pregnant. All pregnant



	<p>females contained viable fetuses. There were no effects of exposure on the number of ovarian corpora lutea, or the number of total, viable or non-viable implantations (early and late resorptions and dead fetuses). Percent pre-implantation and post-implantation losses and sex ratios were equivalent for all groups.</p>
Fetal data:	<p>Fetal examination revealed no evidence of developmental toxicity or fetotoxicity in any of the exposure groups. No exposure-related differences in mean fetal body weights were observed. External, visceral and skeletal examinations of the fetuses revealed no exposure-related differences in the incidences of variations or malformations.</p> <p>Exposure of timed-pregnant rabbits to primary amyl acetate vapors during organogenesis resulted maternal body weight losses during the first week of exposure, reduced maternal food consumption, and reduced maternal body weight gain in females exposed to 1500 ppm. Fetal examination produced no evidence of developmental toxicity or fetotoxicity in any of the exposure groups. The NOAEL for maternal toxicity is 1000 ppm; the NOAEL for developmental toxicity is 1500 ppm.</p>
Reliability:	score = 1, valid without restriction; comparable to GLP guideline study
Reference:	Neeper-Bradley, T.L. and Chun, J.S. 1994 (b). Union Carbide Corporation (unpublished report). Developmental Toxicity Study of Primary Amyl Acetate Vapour in New Zealand White Rabbits. Bushy Run Research Center, Project Report 91U0078, February 3, 1994.
(b) Test substance:	Primary Amyl Acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate
Species:	rat
Strain:	Fischer 344 (CDF F-344 CrI:BR)
Sex:	female
Route of Admin:	inhalation
Exposure Period	gestational days (gd) 6 through 15
Freq. of Treatment:	6 hours/day
Duration of Test:	dams sacrificed on gd 21
Doses:	0, 500, 1000, or 1500 ppm
Control Group:	yes
NOAEL (Maternal Toxicity):	500 ppm
NOAEL (Teratogenicity):	1500 ppm
NOAEL (Develop. toxicity):	500 ppm
Method other:	<p>Four groups, each containing 25 female timed-pregnant F-344 rats were exposed to primary amyl acetate vapor at concentrations of 500, 1000 or 1500 ppm for 6 hours/day on gestational days 6 through 15. Rats were housed in stainless steel wire-mesh cages. All rats were housed separately by test group. During exposures, animals were housed individually, separated by test group in stainless steel wire-mesh cages. Cage positions were rotated daily in a predetermined pattern within each chamber to compensate for any possible but undetected variation in chamber conditions. Food and water was withheld during exposure but available <i>ad libitum</i> at all other times.</p>

Animals were exposed in 1330 L stainless steel and glass inhalation chambers for 6 hours per day. Metering of the liquid primary amyl acetate into a heat glass evaporator generated vapours of the test material. Chamber concentrations were analysed at least once per hour using a gas chromatograph equipped with a flame ionisation detector. All maternal exposures were discontinued from gestation day 16 through study termination.

Clinical observations were conducted daily, and body weights were measured on gestation day 0, 6, 9, 12, 15, 18, and 21. Maternal food consumption was measured at 3-day intervals. All dams were sacrificed on gestation day 21. Ovarian corpora lutea of pregnancy were counted and the status of implantation sites (i.e. resorptions, dead and live fetuses) were identified and recorded. Live fetuses were dissected from the uterus, counted, weighed, sexed and examined for external abnormalities. One half of the live fetuses were examined for visceral malformations and variations, the other half were processed and examined for skeletal malformations and variations.

Data Analysis:

The unit of comparison was the pregnant dam or the litter. The data for quantitative continuous variables were inter-compared for the exposure groups and the control group by use of Levene's test for equality of variances, analysis of variance (ANOVA), and t-tests. The t-tests were used when the F value from the ANOVA was significant. When Levene's test indicated similar variances, and the ANOVA was significant, a pooled t-test was used for pair-wise comparisons. When Levene's test indicated heterogeneous variances, all groups were compared by an ANOVA for unequal variances followed, when necessary, by a separate variance t-test for pair-wise comparisons.

Non-parametric data obtained following laparohysterectomy were statistically evaluated using the Kruskal-Wallis test followed by the Mann-Whitney U test, when appropriate. Incidence data were compared using Fisher's Exact Test. With the exception of the data analysis for fetal malformations and variations, all statistical analyses were performed using BMDP Statistical software (Dixon, 1990). Incidence data for fetal malformations and variations were analyzed using program developed by Bushy Run Research Center. For all statistical tests, the probability value of  $< 0.05$  (two-tailed) was used as the critical level of significance.

Dixon, W.J. 1990. BMDP Statistical Software. University of California Press, Berkeley, CA.

Year:

1994

GLP:

yes

Test substance:

Primary Amyl Acetate, purity 99.1%, mixture composed of 1-pentyl acetate, 65.2%  
2-methyl-1-butyl acetate, 33.9%

Remark:

Exposure of timed-pregnant rats to primary amyl acetate vapour at concentrations of 500, 1000, and 1500 ppm during organogenesis resulted in maternal toxicity at 1500 ppm and reduced or delayed developmental toxicity at vapor concentrations of 1000 and 1500 ppm.

- Result:** Mean daily analytical concentrations of the test material indicated that the variation from the target doses was  $\pm 5\%$  or less. No exposure-related clinical signs were observed during the study in any exposure group. No statistically significant differences in food consumption was observed in any exposure groups during pre-exposure and exposure. Statistically significant decreases in food consumption were observed for all groups exposed to primary amyl acetate during the post-exposure interval.
- Maternal toxicity:** Maternal toxicity was observed in the 1500 ppm group as evidenced by decreases in gestational body weight gain, corrected body weight, and corrected body weight gain. Corrected body weight gains for females in the 1000 and 1500 ppm groups were reduced by 13.3% and 20.4%, respectively, relative to controls. There was no change in food consumption during the exposure interval (GD 6 to 15); however, maternal food consumption was significantly reduced in all treated groups during the post-treatment interval (see table below). No exposure-related necropsy findings were observed at sacrifice on gestational day 21.
- Pregnancy/litter data:** No females aborted or delivered early. All pregnant females contained viable fetuses. There were no effects of exposure on the number of ovarian corpora lutea, or the number of total, viable or non-viable implantations (early and late resorptions and dead fetuses). Percent pre-implantation loss was slightly reduced in treated groups relative to controls. Sex ratios were equivalent for all groups. Litter size was slightly increased in dams exposed to 1500 ppm. A statistically significant decrease in mean female fetal body weight was observed in the 1500 and 1000 ppm group (see table below).

Maternal and Litter Values in Rats Exposed to Primary Amyl Acetate

	Concentration of Primary Amyl Acetate			
	0 ppm	500 ppm	1000 ppm	1500 ppm
Number females on study	25	25	25	25
Number females pregnant	24	24	24	24
Maternal food consumption (gm)				
Pretreatment (GD 0-6)	12.94 $\pm$ 0.89	13.20 $\pm$ 1.17	13.62 $\pm$ 1.41	12.98 $\pm$ 0.69
Treatment (GD 6-15)	13.60 $\pm$ 0.77	13.59 $\pm$ 0.57	13.36 $\pm$ 0.96	13.28 $\pm$ 0.61
Post-treatment (GD 15-21)	16.69 $\pm$ 0.92	15.87 $\pm$ 1.15**	15.97 $\pm$ 0.82**	15.50 $\pm$ 0.84**
Final corrected body weight <sup>1</sup>	180.84 $\pm$ 5.34	177.59 $\pm$ 6.74	177.38 $\pm$ 5.95	175.33 $\pm$ 5.13**
Body weight gain (corrected) <sup>2</sup>	26.05 $\pm$ 5.34	22.93 $\pm$ 6.83	22.66 $\pm$ 5.33*	20.74 $\pm$ 4.69**
Number pups per litter	7.8 $\pm$ 3.28	8.6 $\pm$ 2.65	7.9 $\pm$ 3.22	9.1 $\pm$ 2.74
Total number of pups	187	207	190	219
Fetal body weights per litter (gm)				
Males	4.533 $\pm$ 0.264	4.472 $\pm$ 0.187	4.458 $\pm$ 0.337	4.367 $\pm$ 0.208
Females	4.240 $\pm$ 0.237	4.227 $\pm$ 0.208	4.091 $\pm$ 0.175*	4.061 $\pm$ 0.151*

1: Corrected body weight = body weight at sacrifice minus gravid uterine weight

2: Body weight change (corrected) = corrected body weight – initial body weight

\* Significantly different from control group,  $p < 0.05$ .\*\* Significantly different from control group,  $p < 0.01$ 

- Fetal data:** There was a significant decrease in fetal body weights among female pups exposed to 1000 and 1500 ppm. Among fetuses examined externally, there was no significant difference in the incidence of

malformations between control animals and animals exposed to primary amyl acetate. Among fetuses examined visceraally, there was no significant difference in the incidence of malformations between control animals and animals exposed to primary amyl acetate. Among fetuses examined skeletally, there was no significant difference in the incidence of malformations between control animals and animals exposed to Primary Amyl Acetate (see table below).

**Summary of Variations Observed in Rat Fetuses<sup>1</sup>**

	Concentration of Primary Amyl Acetate			
	0 ppm	500 ppm	1000 ppm	1500 ppm
Total variations				
Number affected pups	149 (79.7%)	158 (76.3%)	159 (83.7%)	175 (79.9%)
Number affected litters	24	24	24	24
External variations <sup>2</sup>				
Number affected pups	83 (44.4%)	75 (36.2%)	91 (47.9%)	105 (47.9%)
Number affected litters	23	23	22	22
Soft tissue variations <sup>3</sup>				
Number affected pups	24 (24.0%)	30 (27.5%)	42 (41.2%)	37 (32.2%)
Number affected litters	14	13	20	18
Skeletal variations <sup>4</sup>				
Number affected pups	87 (100%)	98 (100%)	88 (100%)	104 (100%)
Number affected litters	23	24	23	23

1: Findings are for the number of affected fetuses, followed by percent affected relative to total number of fetuses examined. No findings were significant as compared to controls ( $p \leq 0.05$ )

2: All fetuses were examined for external variations

3: Approximately 50% of each litter were examined for visceraally for soft tissue defects

4: Approximately 50% of each litter were examined for skeletal defects

There was no statistically significant increase in the overall incidence of variations observed in fetuses exposed to Primary Amyl Acetate.

Accompanying the reduced female fetal body weights were increases in one minor fetal skeletal variation at 1000 ppm, and three minor skeletal variations at 1500 ppm. These specific variations were poorly ossified anterior arch of the atlas (1500 ppm), majority of hindlimb phalanges unossified (1000 and 1500 ppm), and thoracic centrum #9 bilobed (1500 ppm).

Although there were two isolated skeletal variations which were statistically increased at 500 ppm, there was no clear dose-response. The incidences observed were comparable to historical controls. Variations in hindlimb phalanges were difficult to interpret (i.e., higher incidence of *all* unossified and lower incidence of *some* unossified in controls as compared to the 500 ppm group but then the majority unossified was increased at higher doses).

## Percent Incidence of Certain Variations

Skeletal Variation	Concentraon of Primary Amyl Acetate				Historical control range <sup>1</sup>
	0 ppm	500 ppm	1000 ppm	1500 ppm	
Anterior Arch of the Atlas					
Poorly ossified	39.1 (F) <sup>2</sup> 73.9(L) <sup>2</sup>	51.0 83.3	53.4 82.6	40.4 100.0*	39-86 74-100
Unossified	2.3 8.7	1.0 4.2	8.0 30.4	6.7 30.4	2-4 9-17
Split	3.4 13.0	3.1 12.5	3.4 13.0	1.0 4.3	1-6 4.5-25
Bilobed	6.9 21.7	3.1 12.5	4.5 13.0	9.6 34.8	0-7 0-22
Hind Limb Phalanges					
Some poorly ossified <sup>3</sup>	67.8 (F) <sup>2</sup> 100 (L) <sup>2</sup>	72.4 95.8	67.0 91.3	67.3 100	30-68 59-100
Majority poorly ossified <sup>3</sup>	4.6 17.4	5.1 20.8	1.1 4.3	4.8 21.7	5-11 17-27
Some unossified	75.9 82.6	76.5 100*	59.1 91.3	62.5 95.7	32-76 53-96
Majority unossified	5.7 17.4	14.3 41.7	21.6 56.5*	20.2 60.9*	6-28 17-61
All unossified	5.7 21.7	3.1 8.3	14.8 43.5	15.4 47.8	6-31 22-50
Thoracic Centra 9-12					
#9 Split	0(F) <sup>2</sup> 0 (L) <sup>2</sup>	0 0	0 0	1.0 4.3	
#9 Bilobed	1.1 4.3	5.1 16.7	6.8 26.1	6.7 30.4*	1-17 4-58
#10 Split	0 0	0 0	0 0	1 4.3	
#10 Bilobed	10.3 34.8	24.5 75.0*	20.5 56.5	14.4 56.5	6-47 24-92
#11 Split	0 0	1.0 4.2	0 0	1.0 4.3	0-1 0-5
#11 Bilobed	28.7 69.6	38.8 79.2	29.5 60.9	33.7 82.6	4-64 12-100
#12 Split	0 0	1.0 4.2	0 0	1.0 4.3	
#12 Bilobed	19.5 56.5	21.4 58.3	17.0 56.5	18.3 47.8	4-47 6-96

1: Historical control data from five studies performed by the same laboratory

2: Top number = % affected fetuses, bottom number = % affected litters

3: Majority = 5 or more phalanges affected; some = 1 to 4 phalanges affected

\*Statistically different from concurrent controls (p &lt; 0.05)

There were two variations that displayed a dose response when evaluated on a percent litter affected basis. However, the data were statistically significant only at the highest dose. These were the external variation, ecchymosis of the head, and the visceral variation, fetal atelectasis (see table below). Based on these results, the NOAEC for external and visceral variations is 1000 ppm.

Percent incidence of visceral and external variations

Variation	Concentration of Primary Amyl Acetate			
	0 ppm	500 ppm	1000 ppm	1500 ppm
Fetal atelectasis	10.0 <sup>1</sup>	10.0	21.5	17.4
	29.2 <sup>1</sup>	25.0	50.0	62.5*
Ecchymosis-head	1.6 <sup>1</sup>	2.4	21.6	17.4
	12.5 <sup>1</sup>	16.6	29.2	41.7*

1: top number = % affected fetuses, bottom number = % affected litters

\*statistically different from concurrent controls,  $p \leq 0.05$ 

- Remark:** Exposure of timed-pregnant rats to Primary Amyl Acetate vapour during organogenesis resulted in maternal toxicity and decreased female fetal body weights at 1000 and 1500 ppm. No exposure-related malformations were observed at any dose level. The NOAEC for maternal toxicity is 500 ppm. Based on reduced fetal body weights and incidence of variations, the NOAEC for developmental toxicity is 500 ppm.
- Reliability:** score = 1, valid without restriction; comparable to guideline study
- Reference:** Neeper-Bradley, T.L. and Chun, J.S 1994(b). Union Carbide Corporation (unpublished report).  
Developmental Toxicity Study of Primary Amyl Acetate Vapour in Fischer 344 Rats. Bushy Run Research Center, Project Report 91U0079, January 21, 1994.

## 5.10 OTHER RELEVANT INFORMATION

### A. Specific toxicities

- (a) **Test substance:** Primary amyl acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate
- Type:** Single-exposure acute neurotoxicity test in rats via whole-body inhalation
- Species:** rat (male and female)
- Strain:** Sprague Dawley
- Concentration:** 0, 500, 1500, 3000 ppm
- Exposure interval:** 6 hours
- Recovery interval:** 8 days
- NOAEL:** 3000 ppm
- Method:** Four groups of 10 male and 10 female Sprague-Dawley CD rats (53 – 61 days old on day of exposure) were exposed to primary amyl acetate vapour at target concentrations of 0, 500, 1500 or 3000 ppm. Animals received a continuous 6-hour exposure during one day designated as Day 1. Rats were subdivided in replicate groups that contained approximately equal representation from each exposure group. Replicates were exposed on different days, and the data pooled across replicates for computation of summary and inferential statistics. The use of replicates facilitated timely neurobehavioral evaluation of animals immediately after exposure.

The exposure session began when vapor generators were turned on and ended when vapor generators were turned off. The test atmosphere was generated by pumping the liquid test material onto a heated evaporator; the resulting vapor was mixed with a high-volume flow of conditioned air that was directed into the exposure chamber. Chambers were composed of stainless steel and glass and operated in a one-pass flow-through mode with airflow sufficient to produce 12 or more air changes per hour. Control chambers received high-volume flow of conditioned air only. Chamber air was automatically drawn from sampling ports and vapor concentrations determined every 30-60 minutes. Airflow rate, temperature, relative humidity, and oxygen concentration were sampled periodically throughout the 6-hour exposure interval.

Rats remained in the chambers for approximately 30 minutes elapsed after termination of exposure to allow for vapor removal. To verify the composition of the test material, two independent techniques, gas chromatography/mass spectrometry (GC/MS), and nuclear magnetic resonance (NMR) were employed.

During exposure, only animals that were visible within each chamber were observed for overt signs of reaction to treatment. Despite this limitation, an attempt was made to score the startle response to an auditory stimulus during exposure. The stimulus was a sound created by sharply striking an object against the wall of the exposure chamber. Observers were not blind to the treatment conditions, and the observer's judgement was relied upon to score the response as absent, diminished, or normal).

Neurobehavioral evaluations after exposure consisted of a motor activity test followed by functional observational battery (FOB). FOB testing was conducted one week prior to exposure, and again on Day 1 and Day 8. Motor activity was assessed one week prior to exposure, and again on Day 1, Day 2, and Day 8. Motor activity testing was initiated approximately 30 minutes after termination of exposure. FOB evaluations began approximately 10-30 minutes after completion of motor activity testing.

For motor activity evaluations, an automated apparatus assessed total activity during the 60-minute test session and for each successive 10-minute interval within the session. The apparatus counted photocell interruptions in a figure-8 enclosure (San Diego Instruments).

Statistical analysis of motor activity: If analysis of covariance (ANCOVA) with pre-exposure motor activity as a covariate were significant ( $\alpha \leq 0.050$ , then t-tests ( $\alpha \leq 0.05$ ) determined which treatment groups differed from controls for the affected day.

Functional observational battery observations were conducted according to Moser and co-workers (Fund. Appl. Toxicol. 11; 189-

206, 1988) and assessed autonomic, CNS excitability, neuromuscular response, sensory-motor response, and other behaviors in exposed and control rats. Each rat was evaluated in three situations: 1) passive examination while rat was inside a cage to which it had been acclimated for 10-30 min; 2) examination during handling; 3) in an open field arena (see table below).

Parameters Examined in Functional Observational Battery

Autonomic	CNS Excitability	Neuromuscular	Sensory- Motor	Other
lacrimation	ease of handling	gait characteristics	response to sharp sound	bizarre behavior
salivation	muscle tone	coordination		diarrhea
pupil constriction	reactivity to handler	surface to air righting	Response to toe or tail pinch	exophthalmia
defecation	tremors/ convulsions	forelimb grip strength		labored breathing
piloerection	arousal level	hindlimb grip strength		change in body weight
palpebral closure	vocalization	rear foot splay		

Forelimb and hindlimb grip strength were measured as the force exerted at the moment the rat released a grid or bar while its body was drawn away from a calibrated device (Chatillon strain gauge). Landing foot splay was determined as the distance between ink or paint impressions made by the rear feet after releasing the rat from a height of 30 cm. Other FOB parameters were scored according to a simple scale (absent, diminished, normal, excessive). Testers were blind with respect to treatment of animals.

Statistical analysis of FOB: Continuous FOB variables (body weight, grip strength, foot splay) were analysed with ANOVA or ANCOVA using pre-test values as covariates, with t-tests or Dunnett's multiple comparisons to examine differences between treatment and the control group for specific time points.

Year: 1997

GLP: no

Test substance: primary amyl acetate, purity 99.7%, mixture of  
64.2% 1-pentyl acetate  
35.5% 2-methyl-1-butyl acetate

Results: Analysis of atmospheric concentrations in test chambers indicated that the deviation was only  $\pm 1\%$  from target concentrations. No mortality occurred and there were no clinical signs of toxicity or differences in body weights or body weight gains between groups. The rapidity of response to the auditory stimulus administered during exposure and the inability to observe all rats within each chamber precluded scoring of individual animals; the observer determined that there was a slight trend towards a reduced acoustic response during the exposure interval, particularly in the 1500 ppm and 3000 ppm groups. The change in acoustic startle response was not considered to be toxicologically significant based on the subtle nature of the effect,



- the inability to observe all exposed animals, the lack of other changes observed during exposure, and the lack of effects in the detailed evaluations performed after exposure. Some differences for body position and palpebral closure was noted during exposure between control and treated groups, but these were attributed to intergroup variation and not treatment related. There were no treatment related changes observed in the functional observational battery (FOB) evaluations or automated motor activity assessments. Under conditions of this assay, the NOAEL for neurotoxicity was at least 3000 ppm.
- Reliability: score = 2, valid with restriction; neurotoxicity assessment did not include CNS histopathology
- Reference: Beyrouty, P. 1997. An Acute Neurotoxicity Study of a Single Inhalation Whole-Body Exposure of n-Amyl Acetate Vapour (with a 2-Week Observation) in the Albino Rat. ClinTrials BioResearch, Project Report No. 97428, BioResearch Laboratories, Ltd., Senneville, Quebec, report dated February 4, 1997.
- (b) Test substance: amyl acetate, isomer composition not specified
- Type: Single-exposure acute neurotoxicity test in mice via whole-body inhalation exposure.
- Species: mouse (male)
- Strain: Swiss
- Concentration: 0, 500, 1000, 2000, 4000 ppm
- Exposure interval: 20 minutes
- Method: Four groups of adult 8 male CFW Swiss albino mice (27-40 g) were exposed to amyl acetate vapour at target concentrations of 0, 500, 1000, 2000 or 4000 ppm. Exposures were conducted using a static system consisting of a sealed 29L cylindrical glass jar with an acrylic lid. The lid was equipped with injection ports and a fan which projected into the chamber above a 15 cm<sup>2</sup> stainless steel mesh platform. During testing, one mouse was placed in the bottom of the chamber, the lid replaced, and a measured amount of test material was injected onto filter paper located on the wire mesh platform. The fan was turned on which volatilised the test material. Vapor concentrations were confirmed by single-wavelength infrared (IR) spectrometry (Miran 1A, Foxboro Analytical). Mean concentrations were within  $\pm 3\%$  of the nominal concentration within 2.5 min after injection and remained within  $\pm 2\%$  of the target dose throughout the exposure. Each animal was exposed only once to a single test substance concentration.
- Sham exposures (air only) in static chambers were conducted once per day for five days prior to actual exposure. During each sham exposure, motor activity was monitored for 30 minutes. Motor activity was measured via two sets of photocells that bisected the static exposure chamber. Interruption of the photobeams were counted. Prior to exposure, mice were trained on the inverted screen test. Each mouse was required to climb to the top of an inverted screen within 10 seconds during three consecutive training tests. The 10-second cutoff was maintained during testing.

Mice were observed during exposure. Measurement of motor activity was initiated immediately after activation of the chamber fan and continued during the entire 20-minute exposure interval. Stimulus reactivity and open field measurements were conducted using a functional observational battery (FOB) test (Bowen, S.E. et al. 1996. *Neurotoxicol. Teratol.* 18: 577-585) which consisted of 21 different measures assessing five domains of behavioral/physiological responses: CNS activity, CNS excitability, autonomic effects, muscle tone/equilibrium, and sensorimotor reactivity. During the last 2 minutes of exposure, mice were scored on posture, arousal, rearing, clonic movements, tonic movements, palpebral closure, gait, and gait abnormalities. After exposure, each mouse was removed from the static chamber and evaluated for ease of removal and handling reactivity. Piloerection, righting reflex, forelimb grip strength, inverted screen test, landing foot splay, approach response, mobility, and response to audible stimulus, touch, and tail pinch were also evaluated.

Data analysis: concentration-effect curves for motor activity data were analysed using analysis of variance (ANOVA) and Turkey post hoc comparisons ( $p \leq 0.05$ ). The procedure for FOB data analysis was similar to a method used by the U.S.EPA (Creason, 1989; Tilson and Moser, 1992), with modification for between-subject data versus repeated measurements. Continuous and count measures were analysed using separate General Linear Model (GLM) procedures (SAS Institute, Cary, NC). Turkey post hoc tests were used to specify differences from control revealed by the overall analysis. Categorical data were analysed with CATMOD (SAS Institute), a procedure designed to provide a model of ANOVA for categorical data. When appropriate, frequencies of behaviors were compared to control frequencies. In addition, analyses were conducted on each domain of solvent effects by performing CATMOD procedures (Moser, 1991). Creason, J.P. 1989. Data evaluation and statistical analysis of functional observational battery data using a linear models approach. *J. Am. Coll. Toxicol.* 8: 157-169.

Tilson, H.A. and Moser, V.C. 1992. Comparison of screening approaches. *Neurotoxicology* 13: 1-14.

Moser, V.C. 1991. Applications of a neurobehavioral screening battery. *J. Am. Coll. Toxicol.* 10: 661-669.

Results:

No mortality occurred during or after exposures. No seizures or biphasic

activity were observed at any concentration. No changes in motor activity were observed. During exposure, mice exposed to 4000 ppm amyl acetate exhibited decreased rearing; recovery was rapid and rearing frequency was normal within 4 minutes after termination of exposure. Mice also displayed increased palpebral closure when exposed to 4000 ppm, possibly related to the irritant characteristic of the test material. After exposure, mice exposed to 4000 and 2000 ppm displayed decrease arousal, increased handling reactivity, and clonic movements. Mice from the 4000 ppm group also displayed increased reactivity to an auditory stimulus and to pain (tail pinch). The

	majority of effects noted were limited to mice exposed to 4000 ppm. These results suggest that amyl acetate has a low order of toxicity but that exposure of mice at or above 2000 ppm can produce acute transient CNS effects.
Reliability:	score = 2, valid with restriction; composition and purity of test substance not specified
Reference:	Bowen, S.E. and Balster, R.L. 1997. A comparison of the acute behavioural effects of inhaled amyl, ethyl, and butyl acetate in mice. <i>Fundamental and Applied Toxicology</i> 35: 189-196.
(c) Test substance:	Primary amyl acetate, mixture of approximately 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate
Type:	Subchronic neurotoxicity test in rats via whole-body inhalation
Species:	rat
Strain:	Sprague-Dawley
Sex:	male and female
Route of admin:	inhalation
Exposure period:	13 weeks
Freq. of Treatment:	6 hours/day, 4-5 days per week
Doses:	0, 300, 600, and 1200 ppm
Control group:	yes
NOAEL:	1200 ppm
LOAEL:	NA
Method:	Four groups of male and female Sprague-Dawley CD rats were exposed to primary amyl acetate vapour by inhalation at target concentrations of 0, 300, 600 or 1200 ppm. Animals were exposed for 13 consecutive weeks, 6 hours per day, 5 days per week except during weeks 4, 8 and 13 when animals were exposed for 4 days to accommodate the schedule for behavioral evaluations. Animals received at least 65 exposures. The control and 1200 ppm groups contained 15 rats per sex. The additional 5 rats per sex were included in the control and 1200 ppm groups for use during a possible post-exposure recovery period. Animals were examined before and after each exposure and a complete detailed clinical examination was performed weekly on each animals. During each exposure, animals were observed for overt signs of reaction to the test atmospheres. Body weights and food consumption were measured weekly. Individual animals were examined for possible changes in behaviour using automated motor activity measurements and a functional observational battery (FOB) during pre-study and once during each of weeks 4, 8 and 13.
	For motor activity evaluations, an automated apparatus assessed total activity during the 60-minute test session and for each successive 10-minute interval within the session. The apparatus counted photocell interruptions in a figure-8 enclosure (San Diego Instruments).
	Statistical analysis of motor activity: If analysis of covariance (ANCOVA) with pre-exposure motor activity as a covariate were

significant ( $\alpha \leq 0.050$ , then t-tests ( $\alpha \leq 0.05$ ) determined which treatment groups differed from controls for the affected day.

Functional observational battery observations were conducted according to Moser and co-workers (Fund. Appl. Toxicol. 11; 189-206, 1988) and assessed autonomic, CNS excitability, neuromuscular response, sensory-motor response, and other behaviors in exposed and control rats. Each rat was evaluated in three situations: 1) passive examination while rat was inside a cage to which it had been acclimated for 10-30 min; 2) examination during handling; 3) in an open field arena (see table below).

Parameters Examined in Functional Observational Battery

Autonomic	CNS Excitability	Neuromuscular	Sensory-Motor	Other
lacrimation	ease of handling	gait characteristics	response to sharp sound	bizarre behavior
salivation	muscle tone	coordination		diarrhea
pupil constriction	reactivity to handler	surface to air righting	Response to toe or tail pinch	exophthalmia
defecation	tremors/convulsions	forelimb strength		labored breathing
piloerection	arousal level	hindlimb strength		change in body weight
palpebral closure	vocalization	rear foot splay		

Forelimb and hindlimb grip strength were measured as the force exerted at the moment the rat released a grid or bar while its body was drawn away from a calibrated device (Chatillon strain gauge). Landing foot splay was determined as the distance between ink or paint impressions made by the rear feet after releasing the rat from a height of 30 cm. Other FOB parameters were scored according to a simple scale (absent, diminished, normal, excessive). Testers were blind with respect to treatment of animals.

Statistical analysis of FOB: Continuous FOB variables (body weight, grip strength, foot splay) were analysed with ANOVA or ANCOVA using pre-test values as covariates, with t-tests or Dunnett's multiple comparisons to examine differences between treatment and the control group for specific time points.

Following completion of treatment, 5 rats per sex per group were perfused *in situ* with fixative and those animals in the control and 1200 ppm groups underwent a microscopic examination of nervous system tissues. A gross examination of respiratory tract tissues was performed on an additional 5 rats per sex per group and these tissues were retained for possible future microscopic examination. The remaining animals, including those designated for a possible post-exposure recovery group were euthanized and discarded.

Year: 1997  
GLP: yes  
Test substance: primary amyl acetate, purity 99.7%, mixture of

Results:	64.2% 1-pentyl acetate and 35.5% 2-methyl-1-butyl acetate There were no mortalities and exposure to up to 1200 ppm primary amyl acetate did not result in overt clinical signs of toxicity or changes in body weight and food consumption. Transient subtle decreases in activity during exposure were noted for the 600 and 1200 ppm groups during the first two weeks of exposure; decreased activity was not observed immediately after exposure. There were no changes in brain weight or length in males or females. A statistically significant decrease in brain width for the 600 ppm females was attributed to intergroup variation as the difference between the groups was very small and the values for females from the control and 1200 ppm groups were comparable. There were no treatment related microscopic lesions in the brains of males or females in the 1200 ppm group and there were no gross or histopathological lesions observed in the nervous system that were attributable to treatment. FOB evaluations, automated motor activity measurements, and neuropathological examination did not reveal a treatment-related effect. The NOAEL for subchronic neurotoxicity for this study was 1200 ppm based on a lack of cumulative neurotoxicity following repeated exposure.
Reliability:	score = 1, valid without restriction; comparable to guideline study
Reference:	Beyrouthy, P. 1997b. A 13-Week Inhalation Neurotoxicity Study by Whole Body Exposure of Amyl Acetate Vapour in the Albino Rat. ClinTrial BioResearch Project, Report No. 97429, Bio-Research Laboratories, Ltd., Senneville, Quebec, September 25, 1997.

## B. Toxicodynamics, toxicokinetics

### B. 1 In vitro studies

(a) Test materials:	1-pentyl acetate 2-methyl-1-butyl acetate
Remark:	1-pentyl acetate and 2-methyl butyl acetate are the components of Primary Amyl Acetate are present in the mixture at concentrations of approximately 65% and 35%, respectively.
Type:	<i>In vitro</i> hydrolysis
Species:	rat
Strain:	Fischer 344
Sex:	male
Test substances:	<sup>14</sup> C 1-pentyl acetate (specific activity 6.9 mCi/mmol), purity 99% <sup>14</sup> C 2-methyl-1-butyl acetate (specific activity 6.9 mCi/mmol), purity 98%.
Reference substances:	<sup>14</sup> C 1-pentyl alcohol (specific activity 6.89 mCi/mmol), purity 99% <sup>14</sup> C 2-methyl-1-butyl alcohol (specific activity 6.86 mCi/mmol), purity 98%.

Analytical chemistry: HPLC was used for the analysis of 1-pentyl acetate and 2-methyl butyl acetate and their corresponding alcohols.  
 Equipment and operating parameters:  
 Waters WISH HPLC Model 710  
 Detector: Radiomatic Flo-One/Beta Radioactivity Flow Monitor Column:  
 Phenomenex Bondclone C18 10  $\mu$   
 Mobile phase: Acetonitrile/water (60%/40%), isocratic  
 Column flow: 1.0 ml/min  
 Column temperature: ambient  
 Scintillant flow: 2.0 ml/min  
 Background: 200 dpm  
 Method: Rat liver homogenates were prepared fresh on the day of each experiment.  
 An F-344 rat was sacrificed by CO<sub>2</sub> asphyxiation, the liver removed, and the carcass discarded. A 20% (w/v) homogenate was prepared by first weighing approximately 10 g of excised liver, which was then cut, minced, and rinsed with 50 mM sodium phosphate buffer (pH 7.4). Minced liver was then added to approximately 40 ml of phosphate buffer (50 mM, pH 7.4) in a 50 ml glass homogenizing tube. The liver was homogenized using a Polytron Homogenizer for approximately 15 seconds, then placed on ice until use. Dilutions (v/v) of the 20% liver homogenate were made using phosphate buffer.

Preliminary tests: The rate of hydrolysis of each test substance was independently determined in rat liver homogenate incubations. The concentration of substrates and rat liver was determined by a series of preliminary tests which optimised incubation conditions. To optimise substrate concentration, the acetate was incubated at concentrations of 0.025, 0.082, 0.138, 0.194, and 0.250 mM in 20% liver homogenate in a 37 degree C water bath with continuous agitation. Each incubation vial was sampled at 0, 5, 15, 30, 45, 60, 75, 90, 105, and 120 minutes and the % of acetate and alcohols in each sample determined by HPLC analysis.

To determine the optimal concentration of liver homogenate, experiments were conducted with liver homogenate concentrations of 0, 0.2, 1.0, 5.0, 10, and 20% using a single concentration of 0.25mM for each acetate. Samples were incubated as described above and sampled at 0, 5, 15, 30, 45, 60, 75, 90, 105, and 120 minutes and the % of acetate and alcohols in each sample determined by HPLC analysis.

The final preliminary experiment was conducted with 1-pentyl acetate and 2-methyl butyl acetate at concentrations of 0.05, 0.0832, 0.250, 0.500, and 0.830 mM incubated with 3% liver homogenates. Samples were processed as described above. In the presence of 3% liver homogenate, essentially all the acetate substrates had been converted to their respective alcohols at all concentrations tested. Based on these results, it was determined to conduct definitive testing in the presence of 2% liver homogenate.

Definitive tests: Approximately 46 ul of a 20% rat liver homogenate was added to 1 ml incubation vials; 414 ul of 50 mM sodium phosphate buffer (pH 7.4) was added, for a final homogenate concentration of 2% (v/v) in a volume of 460 ul. To evaluate the effect of substrate concentration on metabolism,  $^{14}\text{C}$ -1-pentyl acetate or  $^{14}\text{C}$ -2-methyl-1-butyl acetate in DMSO was added to incubation vials at concentrations of 0.10, 0.20, 0.25, 0.50, and 0.75 mM and incubated for 1 minute at 37 degree C. The incubation was stopped by addition of 500 ul methanol.

The effect of incubation time on the metabolism of each acetate was evaluated using a single acetate concentration at time intervals of 0, 0.5, 1.0, 2.0, 5.0, and 10 minutes. The substrate concentrations used were 0.0913 mM and 0.0670 mM for 1-pentyl acetate and 2-methyl butyl acetate, respectively. These concentrations were selected as a result of preliminary tests (see above). The incubation was stopped by addition of 500 ul methanol.

To determine the rate of non-enzymatic hydrolysis, liver homogenates were inactivated by addition of approximately 3% Paraoxon 10 minutes prior to a 1-minute incubation with 0.25 mM  $^{14}\text{C}$ -1-pentyl acetate or  $^{14}\text{C}$ -2-methyl-1-butyl acetate in DMSO. Non-inactivated ("active") liver homogenates received 15 ul phosphate buffer in place of Paraoxon in order to achieve the same final volume.

Upon addition of methanol to the incubations, the samples were centrifuged at approximately 6000 rpm for 5 minutes. A portion of the supernatant was transferred to an HPLC vial equipped with a 300 ul Polyspring<sup>®</sup> insert, and 100 ul of the sample was injected into the HPLC system described above. All study samples were analysed for the presence of either 1-pentyl acetate and its alcohol, 1-pentyl alcohol, or 2-methyl butyl acetate and its alcohol, 2-methyl butyl alcohol. The identity of peaks was confirmed using co-elution with radiolabeled standards in DMSO.

Data Analysis: Lineweaver-Burk plots of inverse substrate concentration ( $\mu\text{M}^{-1}$ ) versus inverse enzymatic hydrolysis velocity [ $\mu\text{moles of substrate metabolised/mg protein/min}^{-1}$ ] were then constructed. Linear regression of these plots was used to determine the Michaelis-Menten, first-order, metabolic (hydrolysis) rate constant ( $K_m$ ) and the maximum velocity ( $V_{max}$ ) of enzymatic hydrolysis for each acetate isomer.

Year: 1993

GLP: no

Result: There was a stoichiometric relationship between the amount of acetate disappearing and the percent of the corresponding alcohol appearing in the incubation (see below).

## Hydrolysis of 1-Pentyl Acetate to 1-Pentyl Alcohol

Time	% Acetate <sup>1</sup>	% Alcohol <sup>3</sup>	% Recovery <sup>4</sup>
0	80.46 <sup>2</sup>	17.57	98.03
30 sec	75.53	24.38	99.91
60 sec	69.99	28.59	95.58

## Hydrolysis of 1-Pentyl Acetate to 1-Pentyl Alcohol

Time	% Acetate <sup>1</sup>	% Alcohol <sup>3</sup>	% Recovery <sup>4</sup>
120 sec	65.32	33.87	99.19
5 min	44.10	51.10	95.20
10 min	29.78	69.65	99.43

1: % 1-pentyl acetate substrate remaining in timed incubations;  
initial substrate concentration 0.0913  $\mu$ M

2: Initial concentration of 1-pentyl acetate is less than 100% due to  
non-enzymatic hydrolysis of the acetate to 1-pentyl alcohol prior to  
addition of liver homogenate

3: % 1-pentyl alcohol appearing in timed incubation

4: % total chromatographed radioactivity

## Hydrolysis of 2-Methyl butyl Acetate to 2-Methyl butyl Alcohol

Time	% Acetate <sup>1</sup>	% Alcohol <sup>3</sup>	% Recovery <sup>4</sup>
0	75.59 <sup>2</sup>	22.27	97.86
30 sec	66.14	32.38	98.52
60 sec	58.00	38.97	96.97
120 sec	46.85	51.69	98.54
5 min	33.57	65.96	99.53
10 min	9.38	89.18	98.56

1: % 2-methyl butyl acetate substrate remaining in timed incubation;  
initial substrate concentration 0.0670  $\mu$ M

2: Initial concentration of 2-methyl butyl acetate is less than 100% due  
to non-enzymatic hydrolysis of the acetate to 2-methyl butyl alcohol  
prior to addition of liver homogenate

3: % 2-methyl butyl alcohol appearing in timed incubation

4: % total chromatographed radioactivity

Incubation with the esterase inhibitor, Paraoxon, resulted in acetate concentrations that remained at time 0 concentrations; thereby demonstrating that the majority of hydrolysis observed was enzymatic. Recovery of total radioactivity was between 95 and 99%.

The calculated Michaelis-Menten, first order, metabolic (hydrolysis) rate constant ( $K_m$ ) and the maximum velocity of enzymatic hydrolysis ( $V_{max}$ ) values for 1-pentyl acetate were approximately 484 micromoles and 0.17 micromoles/mg/min, respectively, while 2-methylbutyl acetate had a calculated  $K_m$  and  $V_{max}$  values of approximately 70 micromoles and 0.09 micromoles/mg/min, respectively.

The results of this study indicate that the most likely route of metabolic degradation of the two isomers in rat liver homogenates is hydrolysis which produces the corresponding alcohol, and that this process is enzymatic.



Remark:	This study demonstrates that the most likely route of metabolism of the components of primary amyl acetate (mixture of 1-pentyl acetate and 2-methyl butyl acetate) in rat liver homogenates is hydrolysis, which produces the corresponding primary amyl alcohols 1-pentyl alcohol and 2-methyl butyl alcohol.
Reliability:	score = 2, reliable with restriction, number of replicates not specified, no statistical analysis performed
Reference:	Morris, E.M. 1993. Union Carbide Corporation (unpublished report). Primary Amyl Acetate. Study of <i>In vitro</i> Hydrolysis of n-Pentyl and 2-Methylbutyl Isomers. Bushy Run Research Center, Project Report 91U0142, March 14, 1993.
(b) Type:	<i>In vitro</i> hydrolysis and substrate specificity of carboxylesterases in respiratory tract tissues from rat, rabbit and hamster.
Species:	Rat, Fischer 344 (male) Rabbit, New Zealand White (male) Hamster, Syrian (male)
Test substances:	The esters tested were acetates of straight-chain C1 through C8 alcohols (1-methyl acetate, 1-ethyl acetate, 1-propyl acetate, 1-butyl acetate, 1-pentyl acetate, 1-hexyl acetate, and 1-octyl acetate). [Note-secondary and tertiary alcohols, as well as $\beta$ -lactones were also tested, but results will not be discussed in this summary]. Among the acetates, 1-pentyl acetate was selected for inter-species and inter-tissue comparisons. All esters and standard were purchased from the Aldrich Chemical Co., and were of the highest purity available, however, the specific purity for each test substance was not provided.
Method:	Animals were killed by carbon dioxide asphyxiation. The nasal, lung, and liver tissues were removed and S-9 homogenates prepared according to the method described by Hadley and Dahl (Hadley, W.M. and Dahl, A. R. 1983. Cytochrome P-450-dependent monooxygenase activity in the nasal membranes of six species. <i>Drug Metab. Dispos.</i> 44: 211-215).  S-9 protein concentrations ranged between 0.7 and 2.5 mg/assay in total volumes of 2-5 ml of 0.01 M Tris buffer at pH 7.4. After equilibrium for 2 min at 37 degree C in a Dubnoff shaker, 50 ul of a substrate ester in ethanol was added. For standards, 50 ul of a 0.5M solution of carboxylic acid in ethanol was added. Controls had 50 ul of substrate added but S-9 was omitted. Solutions were shaken for 10 min at 37 degree C. Reaction was stopped by addition of an equal volumes of isopropanol followed by freezing in liquid nitrogen.  Rates of hydrolysis for each acetate were determined by measuring carboxylic acid residues using ion chromatography. Chemical analysis was performed on a Dionex 2000i ion chromatograph using a separator column designed for organic acids and a suppressor column. The eluting solution was 0.001 N HCl prepared in deionised water.

Quantitative Structure Activity Relationship equations of the log of the rate of hydrolysis of the acetates were obtained using the Hansch program from the Pomona College Medicinal Chemistry Project.

Result:

A series esters of straight chain aliphatic alcohol (methanol, ethanol, propanol, butanol, pentanol, hexanol and octanol) were tested as substrates for carboxyesterases in nasal, lung and liver tissues from male F344 rats, male New Zealand White rabbits, and male Syrian hamsters. Hydrolysis rates increased with carbon number up to C-5 and then decreased. Pentyl acetate was the most readily hydrolyzed substrate in this series and was selected for inter-species and inter-tissue comparisons.

Effect of Chain Length on the Hydrolysis Rates of Acetates<sup>1</sup>

Substrate C# <sup>2</sup>	Hydrolysis <sup>3</sup> Rate $\pm$ SE	Observed <sup>4</sup> Log Kh	Predicted <sup>5</sup> Log Kh
Methyl acetate (C1)	15 $\pm$ 3	1.17	1.21
Ethyl acetate (C2)	30 $\pm$ 3	1.48	1.47
Propyl acetate (C3)	56 $\pm$ 4	1.75	1.70
Butyl acetate (C4)	77 $\pm$ 4	1.89	1.87
Pentyl acetate (C5)	94 $\pm$ 4	1.97	1.94
Hexyl acetate (C6)	64 $\pm$ 4	1.81	1.93
Octyl acetate (C8)	47 $\pm$ 4	1.67	1.63

1: incubated with S-9 from rat ethmoturbينات

2: number of carbons in alcohol chain of acetate ester

3: mean of 3-5 determinations; units are nmol carboxylic acid formed/  
mg S-9 protein/min  $\pm$  SE

4: Log Kh is the logarithm of the hydrolysis rate

5: Predicted value from regression analysis

Hydrolysis of pentyl acetate was compared among different tissues derived from three species. In rats, liver S9 had the most catalytic activity, however, in rabbits and hamsters, the ethmoturbينات equalled or exceeded liver. Lung S9 consistently had less esterase activity than the other tissues, but trachea S9 was nearly as active as nasal and liver tissues.

Rates of Hydrolysis of Pentyl Acetate by Respiratory Tract and Liver Tissue S9 Enzymes<sup>1</sup>

Tissue	Rat	Rabbit	Hamster
Maxilloturbينات	100 $\pm$ 8	76 $\pm$ 13	1100 $\pm$ 101
Ethmoturbينات <sup>2</sup>	120 $\pm$ 5	190 $\pm$ 2	1300 $\pm$ 33
Trachea	110 $\pm$ 10	80 $\pm$ 11	930 $\pm$ 143
Lung	75 $\pm$ 4	47 $\pm$ 2	180 $\pm$ 4
Liver	250 $\pm$ 6	380 $\pm$ 14	1100 $\pm$ 81

1: mean of 3-5 determinations; units are nmol carboxylic acid formed/  
mg S-9 protein/min  $\pm$  SE; data represents maximum observed rates

2: rat ethmoturbinate tissue S-9 from different batch than that used to  
to derive data described in previous table

Remark:

These data support the hypothesis that metabolism of alcohol acetate esters, including pentyl acetate, occurs in the lung and liver, and can also occur extensively in the respiratory tract. Inhaled esters may be largely converted to hydrolysis products in the lung, nasal cavity, and liver.

Reliability: score = 2, valid with restriction; purity of test substances not specified  
 Reference: Dahl, A.R., Miller, S.C. and Petridou-Fischer, J. 1987. Carboxylesterases in the respiratory tracts of rabbits, rats and Syrian hamsters. Toxicology Letters 36: 129-136.

## B. 2 In vivo studies

### (a) Critical study and preferred value

Test material: Primary amyl acetate; vapor mixture of 65.7% 1-pentyl acetate and 34.3% 2-methyl butyl acetate

Species: rat

Strain: Sprague-Dawley

Sex: male

Route of Admin: inhalation

Exposure Period: 90 minutes

Freq. of Treatment: Single

Duration of Test: 90 minutes

Exposure Concentration: 3500 ppm (the chamber is charged with a target concentration of 3500 ppm amyl acetate mixture (approximately 2300 ppm n-pentyl acetate and 1200 ppm 2-methylbutyl acetate) and the concentration drops as the rat inhales the test article. Loss to chamber equipment and external surface of the rat is corrected for.

Control Group: None (biological samples taken prior to exposure).  
 The amount inhaled by the rat (versus deposited on chamber equipment surfaces) is corrected for.

Method: In an effort to understand the respiratory bioavailability of aliphatic alcohols and esters, a whole-body plethysmograph was installed in a gas-uptake chamber. The rat has an indwelling jugular cannula implanted in the jugular vein prior to study start and is placed in the plethysmograph. The plethysmograph (containing the rat) is then placed in the gas-uptake chamber. The leads from the plethysmograph and the venous catheter are exteriorized from the chamber for sample and data collection. The chamber is charged with the primary amyl acetate mixture (described above) by injecting a known volume through a heated injection port and the chamber concentration decay curve is followed by gas chromatography. The concentration of the test material in the exposure chamber was verified and monitored using a gas chromatograph equipped with a flame ionisation detector (GC/FID). The GC/FID was calibrated using a minimum of 5 standard concentrations. In addition, venous blood samples are taken at 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, and 90 minutes. The whole-body plethysmograph is designed to measure (non-invasively) ventilatory movements on conscious rats. By collecting data on ventilatory movements, and chamber and venous blood 1-pentyl acetate and 2-methylbutyl acetate concentrations, respiratory bioavailability determinations can be calculated. Blood samples from five animals were analyzed for 1-pentyl acetate, 1-pentanol, valeric acid, 2-methylbutyl acetate, 2-methylbutanol, and 2-methylbutyric acid concentrations.

Year:	2004
GLP:	no (conducted in spirit of GLP, but not specifically)
Test substance:	primary amyl acetate mixture (65.7% n-pentyl acetate and 34.3% 2-methylbutyl acetate)
Purity:	Spectroscopic grade (>99.9%)
Result:	<p>The blood concentrations of 1-pentyl acetate 1-pentyl alcohol, valeric acid, 2-methylbutyl acetate, 2-methylbutyl alcohol, and 2-methylbutyric acid during the exposure period are reported below. The presence of 1-pentyl alcohol and valeric acid following 1-pentyl acetate inhalation exposure clearly demonstrates that 1-pentyl alcohol and valeric acid were the major metabolites of 1-pentyl acetate metabolism. The presence of 2-methylbutyl alcohol and 2-methylbutyric acid following 2-methylbutyl acetate inhalation exposures clearly demonstrates that 2-methylbutyl alcohol and 2-methylbutyric acid were the major metabolites of 2-methylbutyl acetate metabolism. Blood levels of 1-pentyl alcohol exceeded those of 1-pentyl acetate at the first time point measured (5 minutes into the exposure) with blood concentrations of 36 µM. At the next time point (10 minutes into exposure), the levels of 1-pentyl alcohol in the blood was approximately 2-fold higher (55 µM) than the blood levels of 1-pentyl acetate. 1-Pentyl acetate levels peaked at 10 minutes (32 µM) and declined over the remaining 80 minutes. Chamber concentrations decline from time zero, both due to loss to chamber equipment surfaces as well as uptake by the rat (data not shown). Blood 1-pentyl alcohol levels were approximately 1-3-fold higher than blood 1-pentyl acetate levels from 10 to 25 minutes after the start of the exposure. Valeric acid levels reached levels of 3 µM and were maintained throughout the exposure. These data clearly demonstrate that 1-pentyl alcohol and valeric acid are the major toxicants present following 1-pentyl acetate exposures. Blood levels of 2-methylbutyl alcohol exceeded those of 2-methylbutyl acetate at the first time point measured (5 minutes into the exposure) with blood concentrations of 28 µM. At the next time point (10 minutes into exposure), the levels of 2-methylbutyl alcohol in the blood was approximately 4-fold higher (41 µM) than the blood levels of 2-methylbutyl acetate. 2-Methylbutyl acetate levels peaked at 10 minutes (10 µM) and declined over the remaining 80 minutes. Chamber concentrations decline from time zero, both due to loss to chamber equipment surfaces as well as uptake by the rat (data not shown). Blood 2-methylbutyl alcohol levels were approximately 4-5-fold higher than blood 2-methylbutyl acetate levels from 10 to 25 minutes after the start of the exposure. 2-Methylbutyric acid levels reached up to 17 µM at 25 minutes after the start of the exposure. These data clearly demonstrate that 2-methylbutanol and 2-methylbutyric acid are the major toxicants present following 2-methylbutyl acetate exposures.</p>

n-Pentyl acetate, n-pentyl alcohol, and valeric acid blood levels found following n-pentyl acetate inhalation

Sampling Time (minutes)	n-Pentyl acetate*	n-Pentyl alcohol*	Valeric acid*
0	0	0	0
5	25	36	2
10	32	55	3
15	28	54	3
20	28	59	3
25	25	55	3
30	25	52	3
40	19	48	2
50	21	40	2
60	23	34	2
90	20	24	1

\*mean  $\mu$ M whole blood

2-Methylbutyl acetate, 2-methylbutyl alcohol, and 2-methylbutyric acid blood levels found following 2-methylbutyl acetate inhalation.

Sampling Time (minutes)	2-Methylbutyl acetate*	2-Methylbutyl alcohol*	2-Methylbutyric acid*
0	0	0	0
5	8	28	5
10	10	41	10
15	9	44	14
20	9	47	15
25	9	46	17
30	8	44	17
40	7	39	17
50	7	35	15
60	8	32	14
90	7	23	7

\*mean  $\mu$ M whole blood

Reference: Poet, T. 2004. Unpublished data for Primary Amyl Acetate. Battelle, Pacific Northwest National Laboratory, US Dept. of Energy. For Oxo-Process Panel, Chemstar, American Chemistry Council, Arlington, VA, 22209.

(b) Test substance: 1-butyl acetate  
Remark: 1-butyl acetate is the 4-carbon isomer of 1-pentyl acetate. 1-Pentyl acetate is the major component of Primary Amyl Acetate.. This study demonstrates that the 4-carbon alcohol ester is readily hydrolysed in vivo to its respective alcohol, 1-butyl alcohol.

Species: rat  
Strain: Sprague-Dawley

## 5. TOXICITY

ID: 628-63-7,624-41-9

DATE: 01.07.2006

Sex:	male
Route of Admin:	intravenous
Exposure Period:	Bolus injection into indwelling catheter
Frequency of Treatment:	Single
Duration of Test	Intravenous blood sampling occurred from immediately post dosing until approximately 6 hours after bolus injection
Exposure Concentration	0.28 mmol/Kg
Control Group:	yes, concurrent vehicle
Method:	Preliminary studies were conducted to select dose levels, dose formulations, and sampling times for the definitive studies. N-Butyl acetate in saline with 1% Tween 20 was administered individually to five animals via an indwelling femoral vein catheter. Serial blood samples were collected from an indwelling jugular vein catheter and immediately deproteinized to halt enzymatic activity. Concentrations of n-butyl acetate as well as down stream metabolites (n-butanol, n-butyraldehyde, n-butyric acid) were assayed by an internal standard GC-MS selected ion monitoring method.
Year:	2001
GLP:	yes
Test substance:	1-butyl acetate, purity > 99%
Remark:	In vitro studies have demonstrated rapid hydrolysis of amyl acetates to acetic acid and the corresponding alcohol. Although in vivo validation studies have not been performed for amyl acetate, there is in vivo data for butyl acetate. Following intravenous administration of 1-butyl acetate, blood samples were drawn at 0, 0.5, 1.0, 1.5, 2.5, 5, 10, and 30 minutes post dosing. Analysis of blood samples demonstrated a very rapid hydrolysis of butyl acetate to form 1-butyl alcohol. Peak butyl acetate levels were found at 0.5 minutes, the earliest time point tested after administration. At 10 minutes after dosing, 1-butyl acetate levels were at or below detection levels. Butyl alcohol was found in the 0.5 minute sample, and peak levels of butyl alcohol were found at the 1 minute sampling time. Butyric acid was also found at the 0.5 min sampling interval, and peak levels were noted at the 1 minute time point. This study demonstrates the very rapid hydrolysis of 1-butyl acetate to 1-butyl alcohol, with a half-life measured in seconds.
Reliability:	score = 1, valid without restriction
Reference:	Deisinger, P.J. and English, J.C. 2000. Pharmacokinetics of n-Butyl Acetate and Its Metabolites in Rats After Intravenous Administration. Toxicological Health Sciences Laboratory, Health and Environment Laboratory, Eastman Kodak Company, Roachester, NY. Report TX2000-277, for the OxoProcess Panel, Chemstar, American Chemistry Council, Arlington, VA.
(c) Test substance:	1-butyl acetate
Remark:	1-butyl acetate is the 4-carbon isomer of 1-pentyl acetate. This study demonstrates that the 4-carbon alcohol ester is readily hydrolysed in vivo to its respective alcohol, 1-butyl alcohol.

## 5. TOXICITY

ID: 628-63-7,624-41-9

DATE: 01.07.2006

Species:	rat
Strain:	Sprague-Dawley
Sex:	male
Route of Admin:	intravenous
Exposure Period:	Bolus injection into tail vein
Frequency of Treatment:	Single
Duration of Test	Sampling (sacrifice of animals) occurred from 1.5 to 60 minutes post dosing
Exposure Concentration	30-35 mg/Kg
Control Group:	yes, control animals
Method:	Preliminary studies were conducted to select dose levels, dose formulations, and sampling times for the definitive studies. 1-Butyl acetate in physiological saline was administered individually to 32 animals via a tail vein. Serial sacrifices (5/timepoint) yielded blood and brain samples and were immediately deproteinized to halt enzymatic activity (brain was homogenized). Concentrations of n-butyl acetate as well as down stream metabolites (1-butanol, 1-butyric acid) were assayed by HPLC with radiochemical detection.
Year:	1997
GLP:	yes
Test substance:	radiolabeled 1-butyl acetate, purity > 99%
Result:	Following intravenous administration of 1-butyl acetate, target collection times were 0, 1.5, 2.5, 4, 7, 10, 15, 20, and 60 minutes post dosing. Analysis of these blood and brain samples demonstrated a very rapid hydrolysis of 1-butyl acetate to form 1-butyl alcohol. The $t_{1/2}$ for elimination of 1-butyl acetate from the blood was 0.41 minutes and was detected in brain samples at very low concentrations (mean maximum concentration of 3.8 $\mu\text{g}$ equivalents/gram brain tissue at 1.9 minutes). 1-Butyl alcohol levels were found at higher concentrations in the blood ( $C_{\text{max}}$ 52 $\mu\text{g}$ equivalents/gram at $T_{\text{max}}$ of 2.6 minutes) and in brain tissue ( $C_{\text{max}}$ 79 $\mu\text{g}$ equivalents /gram at $T_{\text{max}}$ of 2.5 minutes) but was also eliminated rapidly from both tissues ( $t_{1/2}$ of 1.0-1.2 minutes). The hydrolysis of 1-butyl acetate in blood and brain tissue was estimated to be 99% complete by 2.7 minutes. This initial study demonstrated a very rapid hydrolysis of 1-butyl acetate, with a half-life measured in seconds. It also demonstrates the rapid appearance and disappearance of the down stream metabolites, 1-butanol and 1-butyric acid. The data from this study was used to support the initial development of a physiologically-based pharmacokinetic (PBPK) model for the butyl series of compounds.
Reliability:	score = 1, valid without restriction
Reference:	Deisinger, P.J. and J. C. English. 2001. The <i>In Vivo</i> Pharmacokinetics of n-Butyl Acetate in Rats After Intravenous Administration. Toxicological Health Sciences Laboratory, Health and Environment Laboratory, Eastman Kodak Company, Rochester, NY. Lab. Proj. ID 94-0306BT01, for the Oxo-Process Panel, CHEMSTAR, Chemical Manufacturers Association, 1300 Wilson Blvd., Arlington, VA, Ref. OXO-22.0-BA-Kodak, as presented in Deisinger, et al., Family

		Approach PBPK Modelling of n-Butyl Acetate and Its Metabolites in Male Rats, Abstract #699, The Toxicologist, Vol. 60, No. 1, March 2001.
(d)	Test substance:	2-methyl-1-propyl acetate (isobutyl acetate)
	Remark:	2-methyl-1-propyl acetate is the 4-carbon structural analog of 2-methyl-1-butyl acetate, the minor component of primary amyl acetate. This study demonstrates that the 4-carbon alcohol ester is readily hydrolysed in vivo to its respective alcohol, 2-methyl propyl alcohol.
	Species:	rat
	Strain:	Sprague-Dawley
	Sex:	male
	Route of Admin:	inhalation
	Exposure Period	90 minutes
	Freq. of Treatment:	Single
	Duration of Test	90 minutes
	Exposure Concentration	2000 ppm (the chamber is charged with 2000 ppm isobutyl (2-methyl propyl) acetate and the concentration drops as the rat inhales the test article. Loss to chamber equipment and external surface of the rat is corrected for).
	Control Group:	None (biological samples taken prior to exposure). The amount inhaled by the rat (versus deposited on chamber equipment surfaces is corrected for).
	Method:	In an effort to understand the respiratory bioavailability of aliphatic alcohols and esters, a whole-body plethysmograph was installed in a gas-uptake chamber. The rat has an indwelling jugular cannula implanted prior to study start and is placed in the plethysmograph. The plethysmograph (containing the rat) is then placed in the gas-uptake chamber. The leads from the plethysmograph and the venous catheter are exteriorized from the chamber for sample and data collection. The chamber is charged with 2000-ppm 2-methyl propyl acetate and the chamber concentration decay curve is followed by gas chromatography. In addition, venous blood samples are taken at 0, 5, 10, 20, 25, 30, 40, 50, 60, and 90 minutes. The whole-body plethysmograph is designed to measure (non-invasively) ventilatory movements on conscious rats. By collecting data on ventilatory movements, and chamber and venous blood isobutyl acetate concentrations, respiratory bioavailability determinations can be calculated. Blood samples from two animals were analyzed for 2-methyl propyl acetate and 2-methyl propyl alcohol concentrations.
	Year:	2003
	GLP:	no (conducted in spirit of GLP, but not specifically)
	Test substances:	2-methyl propyl acetate
	Purity:	Spectroscopic grade (>99.9%)
	Result:	The blood concentrations of 2-methyl propyl acetate and 2-methyl propyl alcohol during the exposure period are reported below. The presence of 2-methyl propyl alcohol following 2-methyl propyl acetate inhalation exposure clearly demonstrates that the alcohol was the major metabolite of acetate metabolism. Blood levels of 2-methyl



propyl alcohol equalled those of 2-methyl propyl acetate at the first time point measured (5 minutes into the exposure) with blood concentrations of 15 µM. At the next time point (10 minutes into exposure), the levels of the alcohol in the blood were 5-fold higher (151 µM) than the blood levels of the acetate. 2-Methyl propyl acetate levels peaked at 15 minutes (62 µM) and declined over the remaining 75 minutes. Chamber concentrations declined from time zero, both due to loss to chamber equipment surfaces as well as uptake by the rat (data not shown). Blood alcohol levels were 2.3 to 9.7-fold higher than blood acetate levels from 10 to 25 minutes after the start of the exposure. These data clearly demonstrate that 2-methyl propyl alcohol is the major toxicant present following exposure to 2-methyl propyl acetate.

**Blood levels following inhalation of 2-methyl propyl acetate**

Sampling Time (minutes)	2-methyl propyl acetate*	2-methyl propyl alcohol*
0	0	0
5	14	15
10	31	151
15	62	143
20	15	146
25	16	101
30	15	34
40	23	40
50	14	44
60	20	24
90	10	5

\*mean µM whole blood

- Reference: Poet, T. 2003a. Unpublished data for Isobutyl Acetate (2-Methyl Propyl Acetate). Battelle, Pacific Northwest National Laboratory, US Dept. of Energy. For Oxo-Process Panel, Chemstar, American Chemistry Council, Arlington, VA, 22209.
- (e) Test material: 1-propyl acetate  
Remark: 1-propyl acetate is the 3-carbon isomer of 1-pentyl acetate. 1-Pentyl acetate is the major component of Primary Amyl Acetate. This study demonstrates that the 3-carbon alcohol ester is readily hydrolysed in vivo to its respective alcohol, 1-propyl alcohol.
- Species: rat  
Strain: Sprague-Dawley  
Sex: male  
Route of Admin: inhalation  
Exposure Period: 90 minutes  
Freq. of Treatment: Single  
Duration of Test: 90 minutes  
Concentration: Exposure concentration 2000 ppm (the chamber is charged with 2000 ppm propyl acetate and the concentration drops as the rat inhales the

	test article. Loss to chamber equipment and external surface of the rat is corrected for).
Control Group:	None (biological samples taken prior to exposure). The amount inhaled by the rat (versus deposited on chamber equipment surfaces is corrected for).
Method:	In an effort to understand the respiratory bioavailability of aliphatic alcohols and esters, a whole-body plethysmograph was installed in a gas-uptake chamber. The rat has an indwelling jugular cannula implanted prior to study start and is placed in the plethysmograph. The plethysmograph (containing the rat) is then placed in the gas-uptake chamber. The leads from the plethysmograph and the venous catheter are exteriorized from the chamber for sample and data collection. The chamber is charged with 2000-ppm propyl acetate and the chamber concentration decay curve is followed by gas chromatography. In addition, venous blood samples are taken at 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, and 90 minutes. The whole-body plethysmograph is designed to measure (non-invasively) ventilatory movements on conscious rats. By collecting data on ventilatory movements, and chamber and venous blood propyl acetate concentrations, respiratory bioavailability determinations can be calculated. Blood samples from six animals were analyzed for propyl acetate, propanol, and propionic acid concentrations.
Year:	2003
GLP:	no (conducted in spirit of GLP, but not specifically)
Test substances:	propyl acetate, spectroscopic grade (>99.9%)
Result:	The blood concentrations of propyl acetate, propyl alcohol, and propionic acid during the exposure period are reported below.

#### Blood level concentrations following propyl acetate inhalation

Sampling Time (minutes)	Propyl acetate*	Propyl alcohol*	Propionic acid*
0	0	0	0
5	17	88	5
10	29	102	6
15	36	110	6
20	31	101	9
25	32	94	15
30	33	85	13
40	25	80	7
50	20	70	4
60	14	49	5
90	6	46	4

\*mean  $\mu\text{M}$  whole blood

The presence of propyl alcohol following propyl acetate inhalation exposure clearly demonstrates that propyl alcohol was the major metabolite of propyl acetate metabolism. Blood levels of propyl alcohol (88  $\mu\text{M}$ ) exceeded those of propyl acetate (17  $\mu\text{M}$ ) at the first time point measured (5 minutes into the exposure). At the next time point (10 minutes into exposure), the levels of propyl alcohol in the

blood were approximately 3-fold higher (102 µM) than the blood levels of propyl acetate (29 µM). Propyl acetate levels peaked at 15 minutes (36 µM) and remained fairly level over the next 15 minutes. Chamber concentrations decreased from time zero, both due to loss to chamber equipment surfaces as well as uptake by the rat (data not shown). Blood propyl alcohol levels were up to 2.5 to 8-fold higher than blood propyl acetate levels from 10 to 90 minutes after the start of the exposure.

These data clearly demonstrate that propyl alcohol is the major metabolite present following propyl acetate exposures, and that blood levels of propionic acid are also increased as a result of propyl acetate exposure.

Reliability: score = 1, valid without restriction

Reference: Poet, T. 2003b. Unpublished data on Propyl Acetate. Battelle, Pacific Northwest National Laboratory, US Dept. of Energy. For Oxo-Process Panel, Chemstar, American Chemistry Council, Arlington, VA, 22209.

- (f) Test substance: Isopropyl acetate
- Remark: Isopropyl acetate is the 3-carbon isomer of 2-methyl butyl acetate. 2-methyl butyl acetate is the minor component of Primary Amyl Acetate. This study demonstrates that the 3-carbon alcohol ester is readily hydrolysed in vivo to its respective alcohol, isopropyl alcohol.
- Species: rat
- Strain: Sprague-Dawley
- Sex: male
- Route of Admin: inhalation
- Exposure Period: 90 minutes
- Freq. of Treatment: Single
- Duration of Test: 90 minutes
- Concentration: 2000 ppm (the chamber is charged with 2000 ppm isopropyl acetate and the concentration drops as the rat inhales the test article. Loss to chamber equipment and external surface of the rat is corrected for).
- Control Group: None (biological samples taken prior to exposure). The amount inhaled by the rat (versus deposited on chamber equipment surfaces is corrected for).
- Method: In an effort to understand the respiratory bioavailability of aliphatic alcohols and esters, a whole-body plethysmograph was installed in a gas-uptake chamber. The rat has an indwelling jugular cannula implanted prior to study start and is placed in the plethysmograph. The plethysmograph (containing the rat) is then placed in the gas-uptake chamber. The leads from the plethysmograph and the venous catheter are exteriorized from the chamber for sample and data collection. The chamber is charged with 2000-ppm isopropyl acetate and the chamber concentration decay curve is followed by gas chromatography. In addition, venous blood samples are taken at 0, 5, 10, 20, 25, 30, 40, 50, 60, and 90 minutes. The whole-body plethysmograph is designed to measure (non-invasively) ventilatory movements on conscious rats. By

collecting data on ventilatory movements, and chamber and venous blood isopropyl acetate concentrations, respiratory bioavailability determinations can be calculated. Blood samples were analyzed for isopropyl acetate and isopropanol concentrations.

Year: 2003

GLP: no (conducted in spirit of GLP, but not specifically)

Test substances: isopropyl acetate

Purity: Spectroscopic grade (>99.9%)

Result: The blood concentrations of isopropyl acetate and isopropanol during the exposure period are reported below. The presence of isopropanol following isopropyl acetate inhalation exposure clearly demonstrates that isopropanol was the major metabolite of isopropyl acetate metabolism. Chamber concentrations decline from time zero, both due to loss to chamber equipment surfaces as well as uptake by the rat (data not shown). Blood levels of isopropyl acetate increased up to 54 µM at 10 minutes into the exposure, and declined over the remaining 80 minutes. Isopropanol blood levels increased up to 268 µM at 50 minutes, after which they declined to 245 µM at 90 minutes. Isopropanol blood levels exceeded isopropyl acetate blood levels at 5 minutes into the exposure, and at every time point thereafter.

Isopropyl acetate and isopropanol blood levels found following isopropyl acetate inhalation

Sampling Time (minutes)	Isopropyl acetate*	Isopropyl alcohol*
0	0	0
5	39	57
10	54	88
15	45	133
20	48	146
25	41	178
30	35	219
40	26	221
50	29	268
60	30	267
90	24	245

\*mean µM whole blood (n=6 for isopropyl acetate)

Reliability: Score=2, valid with restrictions

Reference: Poet, T. 2003c. Unpublished data for Isopropyl Acetate. Battelle, Pacific Northwest National Laboratory, US Dept. of Energy. For Oxo-Process Panel, Chemstar, American Chemistry Council, Arlington, VA.

**C. Other**

(a) Test substances: Acetate esters and aliphatic alcohols (C-1 through C-5)

Remark: The partition coefficients for a series of aliphatic alcohols and acetates (C-1 through C-5) were determined in distilled water, human blood, olive oil and rat tissues.  
Human blood was collected from five health male volunteers (average age 30, mean packed cell volume 51.8%).  
Rats were anesthetized with sodium pentobarbitone and blood collected from the abdominal aorta with a heparinized syringe. The liver, kidneys, muscles, brain, and fat were excised and weighed. All tissues except fat were homogenized with distilled water. The net volume of each tissue was calculated assuming that the specific gravity of tissue was 1.0. The fat tissue was homogenized without water, and 0.5 g portions used for partition measurement. The volume of the fat sample was calculated from its average specific gravity of 0.908.

Blood/air, oil/air, and tissue/air partition coefficients were determined using the vial equilibrium method (Sato, A. and Nakajima, T. 1979. Partition coefficients of some aromatic hydrocarbons and ketones in water, blood, and oil. Br. J. Ind. Med. 36: 231-234).

The coefficients for 1-pentyl alcohol are listed below. Values are means (SD) of five measurements:

Water/air:	1080 (27)	Olive oil/air:	1380 (130)
Human blood/air:	534 (23)	Rat blood/air:	829 (56)
Rat liver/air:	435 (16)	Rat kidney/air:	1100 (120)
Rat fat/air:	2560 (190)	Rat brain/air:	1080 (89)
Rat muscle/air:	814 (85)		

Tissue/blood partition coefficients for 1-pentyl alcohol were nearly all greater than 1. The fat/blood coefficient was almost 40. The authors suggest that 1-pentyl alcohol may uniformly distribute throughout the body:

Rat fat/blood:	3.09
Rat liver/blood:	2.11
Rat kidney/blood:	1.33
Rat brain/blood:	1.30
Rat muscle/blood:	0.98

Reference: Kaneko, T., Wang, P-Y. and Sato, A. 1994. Partition coefficients of some acetate esters and alcohols in water, blood, olive oil, and rat tissues. Occupational and Environmental Medicine 51:68-72.

(b) Remark: The anti-hemolytic effect of 1-pentyl acetate was investigated *in vitro* in rat erythrocytes. Addition of 350 ppm (2.37 mM) to a suspension of rat erythrocyte in saline buffer reduced hypotonic hemolysis by 50% relative to control cells. Addition of concentrations

of 1000 ppm or greater (6.77 mM or greater) afforded 100% protection against hemolysis. The antihemolytic effect was evident only when the acetate was present during the hemolytic event. Treated cells displayed an increase in critical cell volume, indicating that the protective effect was a solvent-induced increase in red blood cell membrane stability.

Reference: Holmberg, B., Jakobson, I., and Malmfors, T. 1974. The effect of organic solvents on erythrocytes during hypotonic hemolysis. *Environmental Research* 7: 193-205.

### 5.11 EXPERIENCE WITH HUMAN EXPOSURE

Test substance: Primary Amyl Acetate, mixture of 65% 1-pentyl acetate and 35% 2-methyl-1-butyl acetate

Type: in vitro percutaneous absorption in human skin

Species: human

Exposure Period: 24 hours

Freq. of Treatment: Single

Duration of Test: 24 hours

Method: OECD Draft Guideline for Dermal Delivery and Percutaneous Absorption: *In vitro* Method, 1996.

Human whole skin samples were obtained post-mortem. The skin samples were immersed in water at 60 degrees C for 40-45 seconds and the epidermis teased away from the dermis. Each epidermal membrane was given an identifying number, placed on aluminum foil, and stored frozen until used.

Samples of epidermis were mounted in five glass diffusion cells with an exposed area of 2.54 cm<sup>2</sup> as described by Dugard and coworkers (1984). The receptor portion of each diffusion cell was filled with a known volume of receptor fluid (50% ethanol in distilled water). Cells were placed in a water bath maintained at a temperature of 32 ± 1 degree C. The integrity of the membranes was determined by measurement of their electrical resistance across the skin membrane. Membranes with a measured resistance of greater than 10 Kohms were considered to have a normal integrity.

Undiluted primary amyl acetate was applied to the skin membranes at a dose of 100 ul/cm<sup>2</sup> and left unoccluded on the skin surface for 24 hours. A sample of the receptor fluid was drawn out of each receptor chamber at 0 (pre-treatment), 0, 10 minutes, and 30 minutes; and at 1, 2, 3, 4, 6, 8, 10, 16, 20, and 24 hours. Samples were extracted into iso-hexane, and then analyzed for each amyl acetate isomer using a gas-liquid chromatograph equipped with a flame ionization detector. Standard solutions of 1-pentyl acetate and 2-methyl-butyl acetate were similarly extracted and analyzed.

Results of sample analysis were expressed as the amount of 1-pentyl acetate and 2-methyl-butyl acetate in the receptor solution in terms of ug/cm<sup>2</sup>. The amounts absorbed, rate of absorption (ug/cm<sup>2</sup>/hr) and permeability coefficient Kp in cm/hr) were also determined.

Year: 2000  
GLP: yes  
Test substance: Primary Amyl Acetate, purity 99.8%  
Results: Both isomers of primary amyl acetate are moderately well absorbed through the human epidermis. Mean total absorption data were presented in tabular form. Total mean absorption of 2-methyl-butyl acetate over 24 hr was approximately 1100 ug/cm<sup>2</sup>. Total mean absorption of 1-pentyl acetate over 24 hr was approximately 2700 ug/cm<sup>2</sup>. The absorption profiles of both isomers were similar, and the absorption rates were essentially constant after the first 6 hours of exposure.

Absorption of Primary Amyl Acetate Isomers through Human Epidermis

Test material applied*	Mean absorption rate (ug.cm <sup>2</sup> /hr ± SEM)	Permeability Coefficient (cm/hr ± SEMx 10 <sup>-4</sup> )
2-methyl butyl acetate 30.65 mg/cm <sup>2</sup> occluded contact 24 hours, n = 5	47.8 ± 8.63	1.56 ± 0.28
1-pentyl acetate 569.2 mg/cm <sup>2</sup> occluded contact 24 hours, n = 5	123 ± 19.4	2.16 ± 0.34

\*100 ul primary amyl acetate applied per cm<sup>2</sup>, amount of each isomer applied determined from concentration in mixture and specific gravity of 0.8757.

Reliability: Score = 1, valid without restriction; guideline study.  
Reference: Ward, R.J. 2000. Primary Amyl Acetate, Mixed Isomers: In vitro Percutaneous Absorption Study in Human Skin. CTL/JV1596 Regulatory Report, Central Toxicology Laboratory, Cheshire, UK.

(b) Remark: The odor detection threshold for primary amyl acetate (>99% purity) was measured in 23 human subjects by means of an olfactometer. The mean olfactory detection threshold was 0.18 +/- 0.03 ppm. The detection threshold for each individual was defined as "the lowest concentration above which the subject detected the odor at each presentation in both runs."

Reference: Laing, D.G. 1982. Characterisation of human behaviour during odour perception. Perception 11: 221-230.

(c) Remark: Eye, nose and throat irritation following exposure to primary amyl acetate was assessed in human subjects. Groups of 10 subjects of both sexes were individually placed in a 1200 cubic foot

- exposure chamber and exposed to different concentrations of amyl acetate vapour. Following each 3 to 5 minute exposure, subjects classified the degree of irritation of the eyes, nose and throat. Amyl acetate caused "mild" eye and nose irritation and "severe" throat irritation at 200 ppm. "Slight throat discomfort" was experienced at 100 ppm.
- Reference: Nelson, K.W., Ege, J.R., Jr., Ross, M. Woodman, L.E., Silverman, L. 1943. Sensory response to certain industrial solvent vapours. *J. Industrial Hygiene and Toxicology* 25: 282-285. *cited in:* Elder, R.L. (ed). 1988. Safety Assessment of Cosmetic Ingredients. Final Report on the Safety Assessment of Amyl Acetate and Isoamyl acetate. *J. American College of Toxicology* 7: 705-719.
- (d) Remark: At 300 ppm in air, the vapour of primary amyl acetate is noticeably irritating to human eyes. At higher concentrations it causes a burning sensation in the eyes and hyperemia of the conjunctiva, but no corneal damage has been observed. Exposure of thirty workers to 20 to 80 mg/liter of air caused initially a sensation of irritation to the eyes to which the workers gradually became accustomed. Examination after chronic exposures showed only hyperemia of the bulbar conjunctiva, and no abnormalities of the cornea, particularly no vacuoles in the corneal epithelium.
- Reference: Grant, W.M. 1986. *Toxicology of the Eye*, 3<sup>rd</sup> edition. Springfield, IL: Charles C. Thomas Publishers, p. 97.
- (e) Remark: Two men exposed to 900 ppm primary amyl acetate for 0.5 hr reported an initial effect of throat irritation and cough, then irritation of the conjunctiva and marked nasal secretion with dryness of the throat and slight fatigue. No other effects were observed.
- Reference: Browning, E. 1965. *Toxicity and Metabolism of Industrial Solvents*. New York: American Elsevier, p. 537-542.
- (f) Remark: Humans exposed to 1000 ppm primary amyl acetate for 0.5 hr complained of headache, fatigue, excessive salivation, and irritation of the mucosal membranes of the eyes, nose, and throat. These effects are usual transitory. Although rare in occurrence, there have been isolated reports of liver injury, gastrointestinal disturbances, and blood changes in workers exposed to the solvent via inhalation.
- Reference: Lehmann, K.G. and Flury, F. 1943. *Toxicology and Hygiene of Industrial Solvents*, p.227. *cited in:* Browning, E. 1953. *Toxicity of Industrial Solvents*. American Elsevier, p. 299.
- (g) Remark: Exposure to primary amyl acetate at levels of 10,000 ppm for 5 hours or more is considered to be potentially lethal to humans.
- Reference: Browning, E. 1965. *Toxicity and Metabolism of Industrial Solvents*. New York: American Elsevier, p. 539.
- (h) Remark: A three-week repeat-insult patch test of 20% primary amyl acetate was conducted using a panel of 211 human subjects. No evidence of delayed contact hypersensitivity was observed and no



- adverse reactions were observed during the entire exposure period to amyl acetate.(See Section 5.3 for robust summary of this study)
- Reference: Cosmetic, Toiletry and Fragrance Association (CTFA). 1987. Submission of unpublished data by CTFA. Repeat insult patch test on amyl and isoamyl acetate. Available for review: Director, Cosmetic Ingredient Review, 1110 Vermont Ave., N.W. Suite 810, Washington, DC 20005. *cited in*: Elder, R.L. (ed). 1988. Safety Assessment of Cosmetic Ingredients. Final Report on the Safety Assessment of Amyl Acetate and Isoamyl acetate. J. American College of Toxicology 7: 705-719.
- (i) Remark: A three-week human photoallergy and primary phototoxicity test on 20% primary amyl acetate was conducted using a panel of 25 human subjects. During the induction period the treatment sites were evaluated and fresh patches applied two times per week for three weeks. There was no evidence of a phototoxic or photoallergenic response in any of the subjects tested. .(See Section 5.3 for robust summary of this study)
- Reference: CTFA. 1987. Submission of unpublished data by CTFA. Clinical photo-toxicity and photosensitization test results. Available for review: Director, Cosmetic Ingredient Review, 1110 Vermont Ave., N.W. Suite 810, Washington, DC 20005. *cited in*: Elder, R.L. (ed). 1988. Safety Assessment of Cosmetic Ingredients. Final Report on the Safety Assessment of Amyl Acetate and Isoamyl acetate. J. American College of Toxicology 7: 705-719.

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