AR201-13033a

The Flavor and Fragrance High Production Volume Consortia

The C_6-C_{10} Consortium

Test Plan for C_6-C_{10} Aliphatic Aldehydes and Carboxylic Acids

Heptanal	CAS No. 111-71-7
Heptanoic acid	CAS No. 111-14-8
Octanal	CAS No. 124-13-O
Nonanal	CAS No. 124-19-6

FFHPVC C_6-C_{10} Aliphatic Aldehydes and Carboxylic Acids Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:

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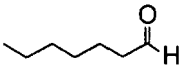
The HPV Challenge Test Plan for C_6-C_{10} Aliphatic Aldehydes and Carboxylic Acids

1 Identity of Substances



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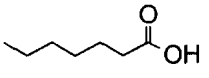

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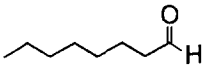

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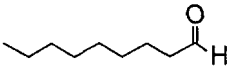


Nonanal
CAS No. 124-19-6

2 Category Analysis

2.1 Introduction

In October of 1999, members of the U.S. flavor and fragrance industries as well as other manufacturers that produce source materials used in flavors and fragrances formed consortia of companies in order to participate in the Chemical Right-to-Know Program. Members of these consortia are committed to assuring the human and environmental safety of substances used in flavor and fragrance products. The consortia are organized as the Flavor and Fragrance High Production Volume Consortia (FFHPVC). The C_6-C_{10} aliphatic aldehydes and carboxylic acids consortium, as a member of FFHPVC, serves as an industry consortium to coordinate testing activities for aliphatic substances under the Chemical Right-to-Know Program. Four (4) companies are current members of the C_6-C_{10} Consortium. The C_6-C_{10} Consortium and its member companies are committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and where needed, conducting additional testing. The test plan, category analysis and robust summaries presented represent the first phase of the Consortium's commitment to the Chemical Right-to-Know Program.

2.2 Background Information

This category analysis and test plan provides data for a group of three (3) saturated aliphatic acyclic linear aldehydes; heptanal, octanal, and nonanal, and one (1) carboxylic acid; heptanoic acid. The four substances in this chemical category are currently recognized by the U.S. Food and Drug Administration (FDA) as GRAS ("generally regarded as safe") for their intended use as flavoring substances [Hall and Oser, 1965]. Two aldehydes, octanal and nonanal, have been reviewed by the Joint Expert Committee on Food Additives (JECFA), a part of the World Health Organization (WHO) in 1984 at the twenty-eighth meeting of the Committee. The Committee established an acceptable daily intake (ADI) of 0.1 mg/kg bw/day for the combined use of octanal and nonanal based on the presumed *in vivo* oxidation to the corresponding acids and the results of a short-term study [JECFA, 1984].

By virtue of the fact that the aldehydes in this category are precursors of short-chain fatty acids or a short-chain fatty acid (heptanoic acid), they are expected to occur naturally in foods. Quantitative natural occurrence data indicate that oral intake of these substances occurs predominantly from consumption of food [Stofberg and Grundschober, 1987; Stofberg and Kirschman, 1985]. Greater than 1,000,000 kg of heptanal, octanal, and nonanal, and their corresponding carboxylic acids are consumed annually as natural components of traditional foods [Stofberg and Grundschober, 1987].

2.3 Structural Classification

The chemical category designated "C_6-C_{10} Straight Chain Aliphatic Aldehydes and Related Carboxylic Acids" includes a homologous series of straight chain saturated aldehydes of carbon chain length C_7 to C_9, heptanal, octanal, nonanal and one structurally related carboxylic acid, heptanoic acid. Heptanal is readily oxidized to heptanoic acid. The four substances are assigned to the same chemical category because of their close structural relationships and their similar physio-chemical properties. The three aldehydes are readily oxidized to their corresponding carboxylic acids in *vivo*. These carboxylic acids are endogenous in animals in that they are formed or broken down in the fatty acid pathway.

Heptanal and octanal are colorless liquids exhibiting a penetrating odor with a citrus-like aroma upon dilution. Nonanal is a colorless liquid with a fatty rose-like aroma. Heptanoic acid, better recognized as enanthic acid, is an oily liquid with a disagreeable, rancid, fatty odor that is faint when very pure [Bauer and Garbe, 1985].

2.4 Metabolism of Straight Chain Saturated Aliphatic Aldehydes and Carboxylic Acids

Linear aliphatic acyclic alcohols [DeBruin, 1976; Lington and Bevan, 1994], aldehydes [Brabec, 1993], and carboxylic acids [Katz and Guest, 1994; Dawson et *al., 1964;* von Oettingen, 1960] are absorbed through the gastrointestinal tract and are rapidly eliminated from the blood. Plasma half-lives are normally difficult to measure since many low molecular weight

aldehydes, and carboxylic acids (e.g., propionic acid) are endogenous in humans [Lington and Bevan, 1994]. Prior to absorption, simple aliphatic aldehydes may undergo oxidation to yield the corresponding carboxylic acid.

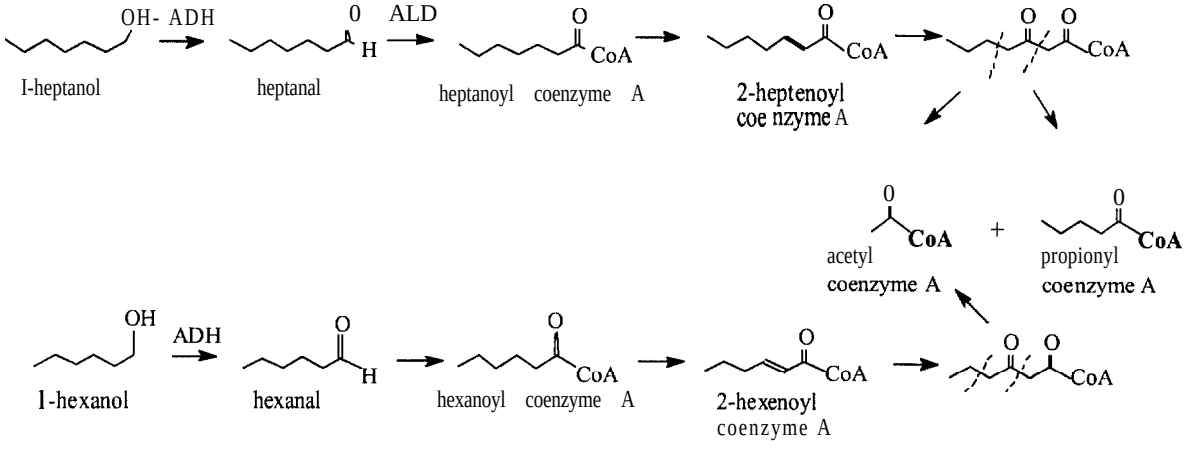
The primary metabolism of linear saturated aliphatic aldehydes and acids is a fundamental part of cell biochemistry. Aldehydes are successively oxidized to their corresponding carboxylic acids. The acid as the coenzyme A (CoA) ester then participates in the fatty acid pathway and the tricarboxylic acid cycle. To a minor extent, aldehydes also may be reduced to alcohols or conjugated with labile sulfhydryl-containing substances, such as glutathione [Brabec, 1993]. Medium chain carboxylic acids are condensed with acetyl CoA to form fatty acids (e.g., C_{10} to C_{18}) or undergo omega-oxidation to form diacids that are further metabolized by *beta*-oxidation in the fatty acid cycle [Katz and Guest, 1994].

A combination of high capacity dehydrogenase {alcohol (ADH) and aldehyde (ALD) dehydrogenase} and oxidase enzymes rapidly oxidize linear saturated aldehydes to the corresponding carboxylic acids (Figure 1). Most active is a NAD^+/NADH-dependent aldehyde dehydrogenase present in the cytosol that exhibits broad specificity for aliphatic and aromatic aldehydes [Feldman and Weiner, 1972]. It has been reported that the activity of this enzyme increases with increasing molecular weight of the aldehyde substrate [Nakayasu *et* al., 1978]. Molybdenum-containing enzymes, xanthine oxidase and aldehyde oxidase, also catalyze oxidation of a wide range of aldehydes to carboxylic acids [Levi and Hodgson, 1989].

The carboxylic acid resulting from oxidation of the aldehyde enters cellular fatty acid metabolism [Voet and Voet, 1990]. The acid is condensed with CoA to yield the corresponding thioester that undergoes dehydrogenation to form the *trans*-2-alkenoyl CoA (trans-Δ^2-enoyl CoA). The *trans*-Δ^2-alkenoyl CoA thioester is enzymatically converted to the S-3-hydroxy thioester and then to the 3-keto thioester. The thioester then undergoes beta-cleavage to yield an acetyl CoA fragment and a new thioester reduced by 2 carbons (Figure 1). Even numbered carbon acids (e.g. hexanoic or octanoic acid) continue to be cleaved to acetyl CoA while odd numbered carbon acids (e.g. pentanoic or heptanoic acid) yield acetyl CoA and propionyl CoA. Acetyl

CoA enters the citric acid cycle directly while propionyl CoA is methylated to R-methylmalonyl CoA, epimerized to the S isomer and finally isomerized to succinyl CoA *via the* action of methyhnalonyl CoA mutase. Succinyl CoA then enters the tricarboxylic acid cycle [Voet and Voet, 1990]. The high capacity species-specific enzymes that catalyze the reactions in these pathways result in rapid conversion of simple aliphatic aldehydes and carboxylic acids into carbon dioxide.

Figure 1. Metabolism of Aliphatic Primary Alcohols, Aldehydes and Carboxylic Acids



There is evidence of the complete metabolism of short-chain fatty acids. Twelve volunteers were given either l-l.5 mmole (2-3 μcurie/dose) of $^{14}C_1$-octanoic acid by oral administration or intravenous administration on separate occasions at least three days apart. Exhaled labeled carbon dioxide was first detected from l-2 minutes after intravenous administration and 2-6 minutes after oral administration. An average of 15.7% carbon dioxide was recovered within 50 minutes of oral or intravenous dosing [Schwabe *et al.*, 1964]. Based on this extensive biochemical data on the fatty acid pathway and citric acid cycle, the linear aliphatic aldehydes and acids in this chemical category are efficiently metabolized to carbon dioxide and water.

2.5 Summary for Category Analysis

Based on the known biochemical fate of straight chain aliphatic aldehydes and carboxylic acid, it is concluded that the aldehyde members of this chemical category undergo functional group oxidation to the corresponding carboxylic acid that is subsequently completely oxidized to carbon dioxide and water in the fatty acid pathway and tricarboxylic acid cycle. The physiochemical properties and low toxic potential of these substances are consistent with their known reactivity and common metabolic fate.

3 Test Plan

3.1 Chemical and Physical Properties

3.1.1 Melting Point

All the substances in this chemical category are liquids at ambient temperature. Calculated melting points for the lowest molecular weight aldehyde, heptanal, and heptanoic acid are 43.3 to 43.7 °C and -7.5 to -8 °C, respectively [Merck, 1997; Burdock, 1995; Arctander, 1969]. Given that the reported melting point for the structurally related homologous aldehyde pentanal is -91.5 °C [Hodgman *et* al., 1960], the melting points of octanal and nonanal are expected to be less than 0 "C.

3.1.2 Boiling Point

The three linear aliphatic aldehydes of this chemical category exhibit experimentally determined boiling points in the range from 152.6 to 191 °C at 760 mm Hg [Food Chemical Codex, 1996; Arctander, 1969; Merck, 1997; Burdock, 1995; Brabec, 1993]. The range of boiling points for linear aliphatic aldehydes having a chain length from C_5 to C_{10} is evenly distributed throughout the range of 103.4 to 207 °C [Hodgman et al., 1960].

While none of the reported boiling points was obtained according to currently recognized guidelines, the consistency of the values reported by five standard reference sources [Food Chemical Codex, 1996; Arctander, 1969; Merck, 1997; Burdock, 1995; Brabec, 1993] confirms their reliability. Likewise, the reported boiling point of 223 °C for heptanoic acid from three sources [Arctander, 1969; Hodgman *et al.,* 1960; Burdock, 1995] confii its reliability as does the fact that lower and higher homologues hexanoic acid (mp. 205.5 "C) and octanoic acid (mp. 239.3 "C) exhibit boiling points equally distributed below and above that for heptanoic acid [Hodgman *et al.,* 1960].

3.1.3 Vapor Pressure

The calculated vapor pressures for the three aliphatic aldehydes according to the MPBPWIN program range from 0.47 kPa (3.5 mm Hg) for heptanal, 0.21 kPa (1.6 mm Hg) for octanal, and 0.075 kPa (0.56 mm Hg) for nonanal at 25 "C. The calculated [FMA] or reported [Brabec, 1993] vapor pressures for heptanal (0.40 kPa or 3 mm Hg at 25 "C) and octanal (0.080 kPa or 0.6 mm Hg at 20 "C) are consistent with those calculated by MPBPWIN. Given the model and experimental data, the vapor pressures for the three aldehydes are in the range from 3.0 mm Hg (heptanal) to 0.05 mm Hg (nonanal). As anticipated, the MPBPWIN-calculated vapor pressure of 0.015 kPa (0.11 mm Hg) for heptanoic acid is significantly lower than the less polar corresponding aldehyde heptanal(0.47 kPa or 3.5 mm Hg).

3.1.4 Octanol/Water Partition Coefficients

The log Kow for heptanal measured by a reverse-phase HPLC method [Eadsforth, 1983] is 2.8, indicating a low potential for bioaccumulation from water. Calculated octanol/water partition coefficients have been determined by two methods [KOWIN and C-QSAR]. Predicted log Kow values for the three homologous aldehydes are from 2.29 for heptanal to 3.27 for nonanal [SRA]. The log Kow values of 2.42 for heptanal and 4.01 for nonanal predicted by C-QSAR [Nishimura, 1994] are consistent with calculated KOWIN values [SRA] and with the measured value of 2.8 for heptanal [Eadsforth, 1983]. The log Kow value of 2.42 for heptanoic acid [SRA] is consistent with that for its homologues, hexanoic acid (log Kow=2.05) and octanoic acid (log Kow=3.03) [SRC]. The log Kow value for heptanoic acid is expected to be lower than the measured Kow of 2.8 for the less polar substance heptanal. Based on these values, log Kow for the three aldehydes are in the range fi-om 2.5-4.0.

3.1.5 Water Solubility

Water solubilities of the four substances in this category have been determined by WSKOWIN. The calculated values for the three aldehydes are in the range from 2274 mg/L for heptanal to 132 mg/L for nonanal at 25 "C. The calculated solubility for heptanoic acid is 5316 mg/L at 25 °C [WSKOWIN] and is reported to be 242 mg/L at 15 °C [Merck, 1997].

3.1.6 New Testing Required

- Partition coefficient measurement for nonanal using an OECD Guideline HPLC reverse phase method to validate calculated values.

3.2 Environmental Fate and Pathways

3.2.1 Photodegradation

The calculated photodegradation half-lives for linear aliphatic aldehydes in this chemical category are in the narrow range from 3.9 hours for nonanal to 4.2 hours for heptanal [AOPWIN]. The half-lives for the aldehydes are shorter than those for the corresponding carboxylic acids. The half-lives for linear aliphatic carboxylic acids are in the range from 13.2 hours for nonanoic acid to18.5 hours for heptanoic acid [AOPWIN]. Generally, carboxylic acids are more stable to photolysis conditions than are the corresponding aldehydes. Structurally, the carboxyl free radical formed by hydrogen abstraction of a carboxylic acid is more stable than the enolic free radical formed by *the* &traction of *alpha* hydrogen of an aldehyde. The photodegradation half-lives of the aliphatic aldehydes and carboxylic acid in this category are estimated to be less than one day.

3.2.2 Stability In Water

None of the four substances in this chemical category are capable of hydrolysis. The volatilization half-lives calculated [HENRYWIN] for the three aldehydes are 5.0 to 5.9 hours for a model river and 5.1 to 5.2 days for a model lake. The volatilization half-lives calculated [HENRYWIN] for the three more polar corresponding carboxylic acids, heptanoic, octanoic, and nonanoic acids, are 14 to 25 days for a model river and 106 to 189 days for a model lake.

3.2.3 Biodegradation

Biodegradation tests have been performed for heptanal, nonanal, a homologous aldehyde, decanal, and a homologous acid, nonanoic acid. Heptanal undergoes ready biodegradation in both a closed bottle assay using an OECD 301D guideline [Watkinson, 1984] and in a modified Sturm test using an OECD 30 1B guideline [Watkinson, 1984]. Heptanal undergoes greater than

60% and 63-74% biodegradation after 10 and 28 days in a closed bottle assay and 53-74% biodegradation after 28 days in a modified Sturm test. Nonanal undergoes 50% and 84% biodegradation after 10 and 28 days, respectively using a OECD 302C protocol [Rudio, 1998a], but was only 29% and 32% biodegraded after 10 and 28 days, respectively, using an OECD 301F protocol [Rudio, 1994b]. Decanal was not ultimately biodegradable (i.e., 49.8%) in 28 days using an OECD 301B test protocol [Quest, 1995]. However, nonanoic acid was readily biodegradable. In a biodegradability test using an OECD 301B guideline, nonanoic acid was 72% biodegraded after 29 days [Comb, 1999]. Based on the data for the three linear aliphatic aldehydes and structurally related carboxylic acid nonanoic acid, it is concluded that the three aldehydes will be readily biodegradable as will heptanoic acid. However, in order to properly evaluate the results of the biodegradation study for short chain fatty acids, it is recommended that heptanoic acid be subjected to a biodegradability study according to a standard OECD protocol.

3.2.4 Fugacity

Transport and distribution in the environment were modeled using Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11 [Mackay, 1991]. The principal input parameters into the model are molecular weight, melting point, vapor pressure, water solubility, and log Kow. When measured values were available, they were used, but when they were not available, calculated data from the EPIWIN series of programs were used. Based on the similar structure and comparable physiochemical properties of the aliphatic aldehydes and carboxylic acids, it is not unexpected that these substances would exhibit similar distribution in the environment.

The model predicts that the three aldehydes are distributed mainly to the atmosphere (>80%). Consistent with water solubility and log Kow data the percentage (17%) of heptanal in water is greater than that for nonanal (5.3%) while nonanal, being more lipophilic, is found in higher percentage in the soil horizon (8.7% versus 2% for heptanal). The more polar substance, heptanoic acid is distributed mainly to the water (76.2%) and soil (17.7%). Based on this

physiochemical model, the ratio for distribution of the three aldehydes between water (2- 17%) and fish (0.00011 to 0.00049%) is greater than three orders of magnitude indicating low bioaccumulation.

The significance of these calculations must be evaluated in the context that the substances in this chemical category are products of plant and animal biosynthesis and are, therefore, ubiquitous in the environment. The model does not account for the influence of biogenic production on partitioning in the environment nor does it take into account the recognized reactivity (*i.e.*, oxidation) of linear aliphatic aldehydes. Therefore, the relevance of fugacity calculations for these substances must be evaluated in the context of these factors.

3.2.5 New Testing Required

- Biodegradability test for heptanoic acid using OECD Guideline 302B

3.3 Ecotoxicity

3.3.1 Acute Toxicity to Fish

A 14-day LC50 value *in Poecilia reticulata* has been reported for each of the three aldehydes in this chemical category [Deneer et *al.,* 1988]. An acute 96-hour LC50 has also been determined for heptanal *in Salmo gairdneri* [Stephenson, 1982]. Additionally, an acute 96-hr LC50 has been determined in juvenile fathead *minnows,* bleak, and *Nitocra spinipes* for each of the three corresponding alcohols, 1-heptanol, 1-octanol, and 1-nonanol [Broderius *et al.,* 1995; Bengtsson *et al.,* 1984]. The three alcohols are readily metabolized to the corresponding aldehydes in animals. ECOSAR calculated 96-hr LC50 values are normally less than measured values for the three aldehydes. A 96-hour LC50 has been reported for heptanoic acid [Bell, 1999].

In a semi-static assay in which test solutions were renewed daily, the 14-day LC50 in *Poecilia reticulata* was 8.8 mg/L for heptanal, 7.89 mg/L for octanal, and 3.10 for decanal [Deneer *et al.*, 1988]. The 96-hour LC50 of heptanal in Rainbow trout is reported to be 12 mg/L

[Stephenson, 1982]. These 14-day LC50 values are approximately the same as calculated 96-hour LC50 values of 8.8 mg/L for heptanal, 6.7 mg/L for octanal, and 4.8 mg/L for nonanal [ECOSAR].

The experimental 96-hour LC50 values for the corresponding alcohols are uniformly higher than those for the aldehydes. In juvenile fathead minnows, the 96-hour LC50 values increased from 37.9 mg/L for heptanol to 5.52 mg/L for nonanol [Broderius et *al.,* 1995]. In wild-caught bleak, 96-hour LC50 values decreased from 45 mg/L for heptanol to 16 mg/L for nonanol and, *in Nitocra spinipes,* the LC50 values decreased from 216 mg/L for heptanol to 25 mg/L for nonanol [Bengtsson *et al.,* 1984].

The calculated 96-hour LC50 value of 389 mg/L for heptanoic acid is almost two orders of magnitude greater than that calculated for heptanal [ECOSAR]. The experimental 96-hour LC50 of greater than 92 mg/L for heptanoic acid in fathead minnows [Bell, 1999] supports the high calculated LC50 for this simple organic acid. Based on 14-day and 96-hour LC50 studies for heptanal, octanal, and decanal and the series of aliphatic alcohols, the 96-hour LC50 values are anticipated to be greater than 10 mg/L, indicating that these aldehydes are of low acute toxic potential to fish. The corresponding acid, exhibiting a 96-hour LC50 approaching 100 mg/L, is even more innocuous. Given the current database of information, it will not be necessary to perform additional tests for this toxicity endpoint.

3.3.2 Acute Toxicity to Aquatic Invertebrates

An experimental 48-hour EC50 of 54 mg/L has been reported for *Daphnia magna* treated with heptanal [Stephenson, 1982]. The EC50 value is the concentration resulting in immobilization of 50% of the *Daphnia magna* after 48 hours. EC50 values would be expected to be less than measured LC50 values for heptanal. ECOSAR calculated LC50 values are available for all members of this chemical category. The calculated 96-hour LC50 values for fish and *Daphnia magna* are in the same order of magnitude (*i.* e., 1- 10 mg/L). For the three aldehydes, the 48-hour LC50 values are in the range from 4.8 mg/L for nonanal to 6.7 mg/L for heptanal. The calculated 48-hour LC50 values for the corresponding carboxylic acids are at

least an order of magnitude greater than the LC50 values for their corresponding aldehydes. The 4%hour LC50 values are in the range from 64 mg/L for nonanoic acid to 429 mg/L for heptanoic acid. The experimental data demonstrates that heptanal exhibits a low potential for toxicity to aquatic invertebrates. However, the QSAR algorithm should be validated by conducting an additional test on nonanal. Assuming the measured values for nonanal in *Daphnia magma* is greater than the calculated value, it will not be necessary to conduct this test on the homologue octanal or the oxidation metabolite of heptanal, heptanoic acid.

3.3.3 Acute Toxicity to Aquatic Plants

In a manner similar to invertebrate toxicity, an experimental EC50 value has been reported for heptanal. The 96-hour EC50 value for heptanal with *Selenastrum capricornutum* is 16 mg/L [Stephenson, 1982]. For the three aldehydes, the calculated 96-hour EC50 concentrations are in the range from 5.3 mg/L for nonanal to 44 mg/L for heptanal. The calculated 96-hour EC50 values for the corresponding carboxylic acids are up to two orders of magnitude greater than the EC50 values for their corresponding aldehydes. The 96-hour EC50 values are in the range from 44 mg/L for nonanoic acid to 429 mg/L for heptanoic acid. Given that experimental data is available only for heptanal, the QSAR algorithm should be validated by conducting an additional test on nonanal. Assuming the measured values for heptanal and nonanal in green algae are consistent and greater than calculated values, it will not be necessary to conduct this test on the homologue octanal or the oxidation metabolite of heptanal, heptanoic acid.

3.3.4 New Testing Required

- Acute toxicity to *Daphnia magna* by OECD guideline 202 for nonanal
- Acute toxicity to algae according to OECD guideline 201 for nonanal

3.4 Human Health Toxicity

3.4.1 Acute Toxicity

All substances in this category exhibit a very low order of acute oral and dermal toxicity in rats. Oral LD50 values for heptanal, octanal, and nonanal are all greater than 5000 mg/kg bw [Moreno, 1974, 1977; Shelanski, 1971; Smyth et *al.,* 1962; Harrison, 1976a]. Dermal LD50 values in the rabbit are also greater than 5000 mg/kg bw [Moreno, 1977; Shelanski, 197 1; Smyth et *al.,* 1962; Harrison, 1976b]. The inhalation LC50 for heptanal in rats is 4.7 mg/L [Berardi, 1989].

LD50 values by injection of 1200 and 600 mg/kg bw were reported for heptanoic and octanoic acid, respectively [Oro and Wretland, 1961]. Also for octanoic acid, oral LD50 values of 1283 mg/kg bw [Smyth *et al.,* 1962] and 10,080 mg/kg bw [Jenner et *al.,* 1964] have been reported in rats and a dermal LD50 value of greater than 5000 mg/kg bw [Moreno, 1977] has been reported in rabbits. The inhalation LC50 values for heptanoic and nonanoic acid in rats are 4.6 and 0.46-3.8 mg/L, respectively [Hoffman, 1990]. Based on the wealth of acute oral and dermal data, three aldehydes and heptanoic acid exhibit a low order of acute toxicity.

3.42 *In* vitro and *In vivo* Genotoxicity

As a group, saturated aliphatic acyclic linear aldehydes and carboxylic acids exhibited consistent negative results in the standard Ames assay (AMS), the unscheduled DNA synthesis test (UDS), cytogenetic assays, and mouse lymphoma assays. Following are separate discussions of the results of *in vitro* genotoxicity assays for aldehydes and carboxylic acids in this chemical category.

3.4.2.1 Heptanal, Octanal, and Nonanal

There was no evidence of mutagenicity for this homologous series of aliphatic aldehydes in studies using established strains of *Salmonella typhimurium (e.g.,* TA98, TA100, TA102, TA104, TA1535, TA1537, and TA1538) with or without S-9 metabolic activation [Jagannath,

1980; Mamett *et* al., 1985; Mortelmans *et* al., 1986; Florin *et al.,* 1980; Zeiger *et al.,* 1992]. Concentrations of up to 3333 µg/plate were used in standard [Florin *et al.,* 1980] and preincubation [Marnett *et al.,* 1985; Mortelmans *et al.,* 1986; Zeiger *et al.,* 1992] protocols. A variation on the standard Ames assay, which was also negative, used preincubation gene mutation induction with S-9 metabolic activation [Mortehnans *et al.,* 1986].

In mutation assays with mammalian cell lines, heptanal, which is negative in the Ames assay, also showed no evidence of an increase in the frequency of mutations when incubated with mouse lymphoma L5 178Y TK± (MLA) cells in the presence or absence of metabolic S-9 activation at concentrations up to 100 or 250 nl/ml, respectively. In the same assay, nonanal showed no evidence of mutagenicity at concentrations up to 25 nl/ml without metabolic activation and weak evidence of mutagenicity (2.2 times control values) with activation but only at cytotoxic concentrations (60 and 120 nl/ml) [Myhr, 198 1].

There was no evidence of unscheduled DNA synthesis when either rat or human hepatocytes were incubated with concentrations up to 100 mM nonanal [Martelli *et al.,* 1994].

In standard cytogenetic assays {i.e., chromosomal aberration (ABS) and sister chromatid exchange (SCE)}, no significant increase in ABS was reported when concentrations up to 100 uM (16,200 ug/plate) of nonanal were incubated with freshly prepared F344 rat hepatocytes [Esterbauer et al., 1990; Eckl et al., 1993]. There was no evidence of an increase in mitotic index or frequency of micronuclei when 16,200 ug/plate of nonanal was incubated with freshly prepared rat hepatocytes [Esterbauer *et* al., 1990; Eckl *et* al., 1993]. Nonanal induced a significant increase in the incidence of SCEs in rat hepatocytes but there was no dose-response relationship [Eckl *et al.,* 1993].

3.4.2.2 Heptanoic Acid and Homologues

There was no evidence of mutagenicity when 150,000 ug/plate of heptanoic acid and 50,000 ug/plate of octanoic acid were incubated using established *strains* of *Salmonella typhimurium* (e.g., TA98, TA100, TA1535, TA1537, and TA1538) with or without S-9 metabolic

activation [Heck et *al.*, 1989]. In the same strains, there was no evidence of mutagenicity for heptanoic acid with or without activation at concentrations up to 5000 or 10,000 ug/plate, respectively [San and Schadly, 1989] or for nonanoic acid with or without activation at 10,000 ug/plate [San and Kruel, 1989]. A modification of the standard Ames assay involving preincubation was also negative for a homologous series of 8 carboxylic acids (C_1, C_2, C_3, C_7, C_{10}, C_{12}, C_{14}, C_{18}) at concentrations of up to 10,000 &plate [Zeiger et *al.*, 1992].

In mutation assays with mammalian cell lines, heptanoic acid, which is negative in the Ames assay, exhibited a slight increase in the frequency of mutations when incubated with mouse lymphoma L5178Y TK± (MLA) cells in the presence of metabolic S-9 activation at concentrations greater than 600 ug/ml. The authors noted that culture conditions of low pH and high osmolality, which may occur upon incubation of mouse lymphoma cells with an acidic substance, have been shown to produce false-positive results in this and other assays. Therefore, they indicated that the results of this study should be interpreted cautiously [Heck et *al.*, 1989].

There was no evidence of unscheduled DNA synthesis when rat hepatocytes were incubated with concentrations up to 1000 nl/ml of heptanoic acid or 300 nl/ml of octanoic acid [Heck et *al.*, 1989].

In an *in vivo* assay with a structurally related heptenal derivative, there was no evidence of mutagenicity when *Drosophila melanogaster* were maintained on 25 mM of 2,6-dimethyl-5-heptenal for 3 days in a sex-linked recessive lethal assay [Wild *et al.*, 1983]. In a standard mouse micronucleus assay, there was no evidence of an increase frequency of micronuclei of bone marrow polychromatic erythrocytes when rats were given single intraperitoneal injections of up to 1540 mg/kg bw of 2,6-dimethyl-5-heptenal [Wild *et al.*, 1983].

3.4.2.3 Conclusions

Based on a weight of evidence approach, linear saturated aliphatic aldehydes and carboxylic acids *are* not mutagenic *in vitro* in the standard Ames assay, nor did they show evidence of

genotoxicity in standard UDS assays, or *in vitro* mutagenicity assays monitoring mitotic index, or in the incidence of micronuclei in rat hepatocytes. Results of assays in mouse lymphoma cells and cytogenetic assays such as ABS were predominantly negative. In addition, there was no evidence of *in vivo* genotoxicity for a structurally related aldehyde. Based on the above results and taking into account the endogenous nature of these substances and their known biochemical fate, it is concluded that the aliphatic linear aldehydes and carboxylic acids in this chemical category exhibit low genotoxic potential.

3.4.3 Repeat Dose Toxicity

3.4.3.1 *Octanal*

In an 86-day dietary study, rats (12/sex/group) were maintained on diets containing a mixture of aldehydes; G8: octanal (4 ppm), G9: nonanal (9 ppm), decanal: (2.2 ppm), undecanal (6 ppm), dodecanal and methyl nonyl acetaldehyde (8 ppm). The diet was calculated to provide an average daily intake of 112 mg/kg bw of aldehyde mixture for 12 weeks. Controls were maintained on an unsupplemented diet. After 12 weeks, urine samples were examined for presence of sugar and albumin, and blood was analyzed for hemoglobin levels. At necropsy, liver and kidney weights were measured and the liver and kidneys were subjected to histopathological examination. Based on measurement of growth, food intake, and efficiency of food utilization, hematological examination, urine analysis, liver and kidney weights, and histopathological examination of liver and kidney tissues, there was no evidence of toxicity associated with administration of the mixture of aldehydes [Trubek, 1958].

3.4.3.2 Heptanal and Heptanoic Acid

Groups (15/sex/dose) of Wistar rats were maintained on diets calculated to provide daily intakes of 0 (control), 9, 37 and 150 mg/kg bw/day of 2,6-dimethyl-5-heptenal for 13-14 weeks. Rats were examined daily for mortality and clinical signs. Water intake and body weight were recorded twice weekly. Food consumption was measured daily. Hematological examination *(i.e.,* hemoglobin concentration, erythrocyte count, packed cell volume and

leucocyte count), blood chemical determinations, and urine analysis (i.e., volume, pH, glucose, blood, bile, ketones and protein) were monitored at week 6 and at the end of the study, At termination, the rats were necropsied and histopathological examinations were performed on 26 tissues. At 150 mg/kg bw/day, a slight decrease in renal concentrating ability was reported at week 6 in males and at week 14 in females. Serum glucose levels of both sexes were elevated as compared to the controls at 150 mg/kg bw/day. The authors considered that the higher hemoglobin concentrations in treated groups were not adverse findings. The cause of the increased serum glucose level at the highest dose is unknown. There was no evidence of histopathology in any of the tissue examined including testes and ovaries. The authors considered the 37 mg/kg bw/day dietary level to be the no observable adverse effect level (NOAEL) [Gaunt *et al.,* 1983].

Groups (10/sex/group) of male and female Sprague-Dawley rats were given dose levels of 0, 300, 1500, or 3000 mg/kg bw of 2,6-dimethyl-5-heptenal [Terrill, 1990a] or 0, 875, 1750, or 3500 mg/kg bw of heptanoic acid [Terrill, 1990b] by gavage in corn oil (10 ml/kg) daily for 29 or 27 days, respectively. In both studies, clinical signs were monitored twice weekly and body weights and food consumption were measured weekly. At necropsy, blood was drawn and clinical chemistry, hematological determinations, and organ weights were measured. A variety of tissues (26) were prepared and preserved in 10% formalin. All tissues fi-om the control and high-dose groups and tissue fi-om the heart, liver, kidneys, and gross lesions from the low- and mid-dose group were embedded in paraffin, stained with hematoxylin and eosin, and examined microscopically.

Based on statistically significant changes in liver and kidney weights and histopathology of these organs at dose levels of 1500 and 3000 mg/kg bw/day, the lowest observable adverse effect level (LOAEL) and the no observable adverse effect level (NOAEL) for 2,6-dimethyl-5-heptenal were concluded to be 1500 and 300 mg/kg bw/day, respectively [Ten-ill, 1990a].

Based on decreased body weights and food consumption, gross lesions of the stomach, and microscopic lesions of the non-glandular region of the stomach at 3500 mg/kg bw/day, the

lowest observable adverse effect level (LOAEL) and the no observable adverse effect level (NOAEL) for heptanoic acid were concluded to be 3500 and 1750 mg/kg bw/day, respectively [Terrill, 1990b]. Except for tissue already discussed, there was no evidence of histopathology of other tissue including the testes and ovaries.

In a 28-day study, groups of SD rats (10/sex/group) were maintained on drinking water containing 1, 10, 100, or 1000 mg/L of hexanal for 4 weeks, Based on water intake data, the estimated average daily intake was 0.1, 0.9, 8.6, or 95.7 m&g bw. A control group received tap water and a vehicle control group received 0.5% Emuphor. Daily clinical observations and weekly measurements of body weight and food and water consumption revealed no significant difference between test and control animals. At termination, brain, heart, liver, spleen and kidneys weights showed no significant difference between test and control groups. Also at termination, hematological examination and clinical chemistry determinations showed normal values. Histopathology was performed on 26 tissues in controls and the highest exposure group revealed no microscopic alterations that could be associated with administration of the test material, No adverse effects were reported in this study [Komsta *et al.,* 1988].

In a 28-day dermal study, 500 mg/kg of heptanal, nonanal, heptanoic acid, or nonanoic acid in mineral oil (25% solution) was applied to the freshly clipped lateral and dorsal areas of groups of male and female New Zealand white rabbits (5/sex/group) daily for 5 days per week for 2 weeks. The skin of half the animals was abraded prior to the first, sixth, and eighth dose. A control group received mineral oil only. Viability was recorded twice daily, observations for skin irritation were made daily, and body weights were measured weekly. After 2 weeks, 6 animals (3 with abraided and 3 with intact skin) were necropsied with the remaining 4 animals sacrificed after an additional 2-week recovery period. Tissues from 29 organs were removed and preserved in 10% formalin. In all test groups, most animals exhibited a temporary weight loss after one or two weeks. Most animals treated with heptanal or nonanal showed slight to moderate erythema at the application site during the first week. Localized necrosis and exfoliation occurred in most animals during the second week. Microscopic evaluation revealed epidennal necrosis, epidermal hyperplasia, and hyperkeratosis at the application site. The skin

application sites of animals held to week 4 appeared healed. The sites were re-epithelialized and continuous with normal follicular structure and population. No other microscopic alterations were reported for any other tissue that could be related to administration of the test material [Auletta, 1981].

In an 80-week skin painting study, 50 mg of heptanoic acid was applied to the clipped backs of groups (50) of male C3H/HeJ mice. Negative control groups (50/group) were either untreated or treated with a mineral oil vehicle. A positive control group (50) was treated topically with 0.05% benzo(a)pyrene in mineral oil. Based on survival and body weight data, clinical observations, skin condition, and gross and histopathological examination, there was no evidence of significant toxicity or carcinogenicity in mice treated with heptanoic acid [Suskind, 1985].

Based on the NOAELs obtained in oral studies using an aldehyde structurally related to heptanal [Gaunt et *al.*, 1983; Ten-ill, 1990a], a mixture of linear aliphatic aldehydes [Trubek, 1958], heptanoic acid [Terrill, 1990b], and hexanal [Komsta *et al.*, 1988], it is concluded that the three aldehydes and heptanoic acid exhibit a low order of oral toxicity. The lowest NOAEL of 37 mg/kg bw/day is for an unsaturated aldehyde 2,6-dimethyl- 5-hetpenal that is anticipated to be more toxic than any aliphatic linear saturated aldehyde in this category. Data for hexanal, 2,6-dimethyl-5-heptanal in a second study, and the aldehyde mixture indicates that a NOAEL in excess of 300 mg/kg is expected for each aldehyde in the category. Heptanoic acid exhibits an even lower oral toxicity with a NOAEL exceeding 1000 mg/kg bw/day [Ten-ill, 1990b]. There is also low potential for dermal toxicity based on a dermal study using a 500 mg/kg bw/day dose level of heptanal, nonanal, heptanoic acid, or nonanoic acid for 28 days [Auletta, 198 1].

3.4.4 Reproductive Toxicity

A significant amount of data is derived from reproductive/developmental screening studies performed on heptanoic acid and a structurally related heptenal derivative. Although these studies do not meet OECD guideline standards for reproductive or developmental toxicity, they provide an extensive data set that in combination with the lack of histopathology in reproductive

organs of animals in repeat dose studies, indicate a low potential for reproductive or developmental toxicity.

Four groups of 10 virgin Crl CD rats were administered oral dose levels of 0, 200, 1000, or 2000 mg/kg bw of heptanoic acid or 0, 300, 1500, or 3000 mg/kg bw/day of 2,6-dimethyl-5-heptenal by gavage once daily, 7 days prior to cohabitation, through cohabitation (maximum of 7 days), gestation, delivery, and a 4-day post-parturition period. The duration of the study was 39 days [Vollmuth et al., 1995]. Maternal indices monitored included twice daily observation, measurement of body weights, food consumption, duration of gestation, and fertility parameters (mating and fertility index, gestation index, number of offspring per litter). Offspring indices included daily observation, clinical signs, examination for gross external malformations, and measurement of body weight.

In the heptenal study, clinical signs at 1500 and 3000 mg/kg in dams included significant ($P<0.05$ to <0.01) decreases in body weight and absolute and relative food consumption during the premating period. Eight rats of 10 in the high dose group were moribund or found dead on days 2, 3, and 4 of the premating period. Maternal body weights were decreased during gestation for the mid- and high-dose groups of dams.

Decreased body weights and absolute and relative food consumption in the 300 mg/kg bw/day group occurred only during premating and were not considered adverse effects. One of the two surviving high-dose dams delivered a litter that died during the 4-day lactation period. Mating and fertility at the high dose were similar to controls. Measurements of mating success and fertility were similar for controls, low-dose and mid-dose groups. Significant ($P<0.05$ to <0.01) decreases in pup viability occurred for mid-dose and high-dose groups as compared to controls. The mid-dose litters were significantly less ($P<0.05$) than control group litters. High-dose litters weighed markedly less than controls. No changes in averages for duration of cohabitation or gestation, inplantation sites or pup sex ratios were seen at any dose levels. No malformations or gross lesions in pups were attributable to administration of the test material. Based on the significant decrease in ($P<0.05$) pup weight at birth and pup viability in the mid-

dose group, the NOAEL for the F1 offspring was reported to be 300 mg/kg bw/day. The dose level of 300 mg/kg bw/day had no adverse effects on the reproductive performance of female Sprague-Dawley rats or the growth or development of their offspring [Vollmuth *et al.*, 1995].

In the heptanoic acid study, one and 3 deaths were reported in the 1000 and 2000 mg/kg bw/day dose groups, respectively. Clinical signs at 200 mg/kg bw/day in dams during premating and gestation included a significant increase in r-ales ($P<0.01$). This effect was not reported during the lactation period. In the 1000 and 2000 mg/kg bw/day dose group, significant increases in the incidence of rales ($P<0.01$), and excess salivation ($P<0.01$) were reported during premating and gestation. Excess salivation continued during lactation in the high-dose group. The 2000 mgikg bw/day group showed reduced body weight gains during premating, and significantly ($P<0.05$ to <0.01) decreased average maternal body weights on days 10 and 16 of gestation. Average and relative food consumption was reduced in the high-dose group of dams throughout the study. The high-dose was also associated with reduced mating and fertility that were related to mortality. The duration of cohabitation and fertility and gestation indices at 200, 1000, or 2000 mg/kg bw/day were not different from comparable indices in the control group. The high-dose group exhibited reduced pup weights on day 4 post-parturition. No biologically relevant or statistically significant differences in the number of implantations, duration of gestation, the percentage of dams delivering one or more live pups, and the pup viability index were observed. No malformations or gross lesions were observed in pups at any dose levels. The authors concluded that the dose level of 200 mgikg bw/day of heptanoic acid had no significant adverse effects on the reproductive performance of female Sprague-Dawley rats or the growth or development of their offspring [Vollmuth *et al.*, 1995].

Young female Wistar rats (10) were mated with males and the mating success was monitored by daily vaginal smears. Females were maintained on Purina Dog Chow and water ad libitum. Upon insemination, females were given daily oral doses of 2050 mg/kg bw/day of heptanal for 20 days. Body weights were measured daily and the difference in weight between the weight on the day of insemination, and immediately after parturition were also recorded. Based on the observation that there were no resorptions observed in any of the tested females, the authors

concluded that oral administration of 2050 mg/kg bw/day of heptanal resulted in no evidence of reproductive toxicity in female Wistar rats [Carruthers and Stowall, 1941].

3.4.5 Developmental/Teratogenicity Toxicity

Three linear aliphatic carboxylic acids were administered by gavage in corn oil to groups of pregnant Sprague-Dawley rats in a study designed to investigate the effect of aliphatic acid structure on developmental toxicity. Groups of rats received 100 or 133.3 mg butyric acid/kg bw, 75 or 100 mg pentanoic acid/kg bw, or 1125 or 1500 mg octanoic acid/kg bw daily by tracheal intubation on days 6 to 15 of gestation. Dams were allowed to deliver, and litters were examined through post-natal day 6. With varying degree, both dose levels of the three carboxylic acids resulted in an increase in mortality, a decrease in body weight gain and respiratory distress in treated females. With the exception of a significant decrease ($P<0.05$) in the number of live pups reported at the highest dose level (1500 mg/kg bw) with octanoic acid, there was no other evidence of fetotoxicity, developmental toxicity, or teratogenicity associated with administration of the three carboxylic acids [Narotsky et *al.*, 1994].

Pregnant NMRI mice (15/group) were given a single subcutaneous injection of 0 or 600 mg/kg bw of octanoic acid on day 8 of gestation. Dams were sacrificed on day 18 of gestation and examinations were performed for implantation sites. Each live fetus was individually weighed and inspected for the presence of neural tube defects. There was a non-statistically significant increase in embryolethality (15% in test group versus 7% in controls) and no effect of the test material on fetal weigh or on percentage of exencephaly in live fetuses. There was no evidence of embryotoxicity, teratogenicity, or fetal weight retardation [Nau and Loescher, 1986].

Xenopus embryos were collected following hormone-induced breeding. Each group of 25 embryos were exposed to a control, or 8 concentrations of heptanoic, octanoic, or nonanoic acid. Each acid was tested three times and data were pooled to calculate 96- hour LC50 (lethality), 96-hour EC50 (malformation), and to determine a development hazard index (DHI). For heptanoic, octanoic and nonanoic acids, the 96-hour LC50 values were 319.6, 127.1 and 32.7 mg/L, respectively; the 96-hour EC50 values were 51.3, 28.1, and 6.5 mg/L, respectively;

and the DHI was 6.2, 4.5 and 5.0. The authors noted that a DHI of less than 5.0 indicates a low developmental hazard while a value of greater than 5.0 indicates a moderate developmental hazard [Dawson *et al.,* 1996].

In an embryo/fetal toxicity and teratogenesis study, groups of 22 pregnant Sprague-Dawley rats were given 0 (corn oil vehicle), 1000 mg/kg bw of heptanoic acid, or 1500 mg/kg bw of nonanoic acid on days 6- 15 of pregnancy. Measurement of maternal body weights and food consumption and gross pathology revealed no evidence of maternal toxicity. Measurement of mean ovarian weight, uterine weight, litter size, pregnancy rates, corpora lutea, implantation sites, implantation efficiency, fetal viability, fetal size and sex, gross pathology, and visceral and skeletal examinations of fetuses revealed that there was no evidence of embryo toxicity, fetal toxicity, or teratogenesis related to administration of either heptanoic acid or nonanoic acid [Serota, 1983].

A single dose of 2700 mgikg bw of octanoic acid was administered to female Sprague-Dawley rats undiluted by oral gavage on the morning of day 12 of gestation (day0 = morning of finding vaginal plug). At day 20 of gestation, the rats were killed by chloroform overdose, and survivability, number of implantation sites, and mean fetal weight were recorded. Octanoic acid was devoid of embryotoxic effects except for a slight reduction of fetal weight that may be attributable to the severe maternal toxicity observed at the 2700 mg/kg dose [Scott *et al.*, 1994].

Based on the lack of histopathology of reproductive organs in repeat dose studies, the lack of significant reproductive or developmental effects in the absence of maternal toxicity in two reproductive/developmental screening studies, and the lack of developmental or fetotoxicity in studies with structurally related carboxylic acids, it is concluded that members of this chemical category show no significant evidence of either reproductive or developmental toxicity.

3.4.6 New Testing Required

None

3.5 Test Plan Table

Chemical	Physical-Chemical Properties				
	Melting Point	Boiling Point	Vapor Pressure	Partition Coefficient	Water Solubility
CAS No. 111-71-7 Heptanal	A	A, R	A, Calc	A, Calc	Calc
CAS No. 124-I 3-O Octanal	NA	A	Calc, R	Calc	Calc
CAS No. 124-19-6 Nonanal	NA	A, R	A, Calc	Test, Calc	Calc
CAS No. 111-14-8 Heptanoic acid	A	A, R	A, Calc	Calc	A, Calc

Chemical	Environmental Fate and Pathways			
	Photodegradation	Stability in Water	Biodegradation	Fugacity
CAS No. 111-71-7 Heptanal	Calc	Calc	A	Calc
CAS No. 124-I 3-O Octanal	Calc	Calc	R	Calc
CAS No. 124-I 9-6 Nonanal	Calc	Calc	A, R	Calc
CAS No. 11 l-l 4-8 Heptanoic acid	Calc	Calc	Test, R	Calc

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	Acute Toxicity to Aquatic Invertebrates	Acute Toxicity to Aquatic Plants
CAS No. 111-71-7 Heptanal	A, Calc, R	A, Calc	A, Calc
CAS No. 124-13-O Octanal	A, Calc, R	R, Calc	R, Calc
CAS No. 124-19-6 Nonanal	Calc, R	Test, R, Calc	Test, R, Calc
CAS No. 111-14-8 Heptanoic acid	A, Calc	Calc	Calc

Chemical	Human Health Data					
	Acute Toxicity	Genetic Toxicity *In Vitro*	Genetic Toxicity *In Vivo*	Repeat Dose Toxicity	Repro-ductive Toxicity	Develop-mental Toxicity
CAS No. 111-71-7 Heptanal	A	A	R	A, R	A, R	R
CAS No. 124-13-O Octanal	A	A	R	R	R	R
CAS No. 124-19-6 Nonanal	A	A	R	A, R	R	R
CAS No. 111-14-8 Heptanoic acid	A	A	R	A	A	A, R

Legend

Symbol	Description
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties
0	Other

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