NICNAS

National Industrial Chemicals Notification and Assessment Scheme

Limonene

Priority Existing Chemical Assessment Report No. 22

May 2002

ISBN 0 642 50190 4

Preface

This assessment was carried out under the National Industrial Chemicals Notification and Assessment Scheme (NICNAS). This Scheme was established by the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the IC(NA) Act), which came into operation on 17 July 1990.

The principal aim of NICNAS is to aid in the protection of people at work, the public and the environment from the harmful effects of industrial chemicals.

NICNAS assessments are carried out in conjunction with Environment Australia and the Therapeutic Goods Administration, which carry out the environmental and public health assessments, respectively.

NICNAS has two major programs: the assessment of the health and environmental effects of new industrial chemicals prior to importation or manufacture; and the other focussing on the assessment of chemicals already in use in Australia in response to specific concerns about their health and/or environmental effects.

There is an established mechanism within NICNAS for prioritising and assessing the many thousands of existing chemicals in use in Australia. Chemicals selected for assessment are referred to as priority existing chemicals.

This priority existing chemical report has been prepared by the Director NICNAS in accordance with the IC(NA) Act. Under the IC(NA) Act manufacturers and importers of priority existing chemicals are required to apply for assessment. Applicants for assessment are given a draft copy of the report and 28 days to advise the Director of any errors. Following the correction of any errors, the Director provides applicants and other interested parties with a copy of the draft assessment report for consideration. This is a period of public comment lasting for 28 days during which requests for variation of the report may be made. Where variations are requested the Director's decision concerning each request is made available to each respondent and to other interested parties (for a further period of 28 days). Notices in relation to public comment and decisions made appear in the *Commonwealth Chemical Gazette.*

In accordance with the IC(NA) Act, publication of this report revokes the declaration of this chemical as a priority existing chemical, and therefore manufacturers and importers wishing to introduce this chemical in the future need not apply for assessment. However, manufacturers and importers need to be aware of their duty to provide any new information to NICNAS, as required under section 64 of the IC(NA) Act.

For the purposes of section 78(1) of the IC(NA) Act, copies of assessment reports for new and existing chemical assessments may be inspected by the public at the library of the National Occupational Health and Safety Commission (NOHSC). Summary Reports are published in the *Commonwealth Chemical Gazette,* which are also available to the public at the NOHSC library.

Copies of this and other priority existing chemical reports are available on the NICNAS web site. Hardcopies are available from NICNAS either by using the order form at the back of this report, or directly from the following address:

GPO Box 58

Sydney

NSW 2001

AUSTRALIA

Tel: 1800 638 528

Fax: +61 (02) 8577 8888

Other information about NICNAS (also available on request and from the NICNAS web site) includes:

- NICNAS Service Charter;
- information sheets on NICNAS Company Registration;
- information sheets on Priority Existing Chemical and New Chemical assessment programs;
- safety information sheets on chemicals that have been assessed as priority existing chemicals;
- details for the NICNAS Handbook for Notifiers; and
- details for the *Commonwealth Chemical Gazette*.

More information on NICNAS can be found at the NICNAS web site:

http://www.nicnas.gov.au

Other information on the management of workplace chemicals can be found at the web site of the National Occupational Health and Safety Commission:

http://www.nohsc.gov.au

Overview

Limonene and its isomers were declared Priority Existing Chemicals for full assessment on 1 August 2000 due to widespread use of the chemicals and their reported effects in the liver and kidneys of animals after repeated exposure and skin and eye irritation.

Greater than 100 tonnes of *d*-limonene is manufactured in Australia per year from orange oils by extraction through distillation. *d*-Limonene, dipentene and products containing limonene and its isomers (*d*-, *l*-, *dl*-) are also imported into Australia at about 1600 tonnes a year. The concentration of limonene in products varies widely, from very low to 90%.

d-Limonene and dipentene are mainly used in the formulation of a range of fragrance or flavour blends that are used in the further formulation of end products for industry and consumer use and as food additives. They are also used in the formulation of a large range of industrial and consumer products, mainly cleaning and degreasing products, as replacements for chlorinated solvents. Some pure *d*-limonene and dipentene are also used directly in cleaning and degreasing, removing wax, microbiological and gemstone testing and as a general reagent in laboratories.

Limonene products are either used directly or further diluted or mixed with other components by industrial end users. Industrial products containing limonene are mainly hand cleaners, industrial cleaning/degreasing products and removers and strippers. The final concentration of limonene in industrial products varies widely and ranges from < 1% to 95%. The major use of limonene in consumer products is as flavouring and/or fragrance agents in food, pharmaceuticals and household and cosmetic products. Limonene is also present in essential oils that are used widely in Australia. The concentration of limonene in the final consumer products also varies largely and is predominantly low ≤ 1%, but can be as high as 70%.

Occupational exposure may occur during the use, transportation and disposal of limonene or limonene products. A high potential for worker exposure during use of limonene or limonene products has been identified due to widespread end uses, modes of applications and lack of control measures at some worksites. Workers are likely to be exposed by skin and eye contact during manual operations and cleaning of equipment, repacking, formulation and end use. There is also a potential for inhalation exposure, especially during use of limonene or limonene products in confined spaces and in places with limited ventilation, heated blending processes, high speed mechanical stirring, worksites with an open mixing process and no exhaust ventilation, and certain modes of applications such as spraying. Deliberate skin contact occurs during use of hand cleaners containing limonene.

Limonene is a flammable liquid and explosive vapour/air mixtures may be formed at temperatures above 48°C. Its packing group (III) indicates that flammability risk is in the lower range. Rags or other combustible material that have been dipped or soaked in limonene may spontaneously combust. Distillation to dryness may also lead to concentration of peroxides and the risk of explosion.

d-Limonene is readily absorbed by inhalation and ingestion. Dermal absorption is reported to be lower than by the inhalation route. *d*-Limonene is rapidly distributed to different tissues in the body, readily metabolised and eliminated primarily through the urine.

Limonene exhibits low acute toxicity by all three routes in animals. Limonene is a skin irritant in both experimental animals and humans. Limited data are available on the potential to cause eye and respiratory irritation. Autoxidized products of *d*-limonene have the potential to be skin sensitisers. Limited data are available in humans on the potential to cause respiratory sensitisation.

Autoxidation of limonene occurs readily in the presence of light and air forming a variety of oxygenated monocyclic terpenes. Risk of skin sensitisation is high in situations where contact with oxidation products of limonene occurs.

Renal tumours induced by limonene in male rats are thought to be sex and species specific and are not considered relevant to humans. Repeated exposure affects the amount and activity of liver enzymes, liver weight, blood cholesterol levels and bile flow in animals. Increase in liver weight is considered a physiological adaptation as no toxic effects on the liver have been reported. From the data available, it is not possible to identify a NOAEL for these effects. Limonene is neither genotoxic nor teratogenic nor toxic to the reproductive system.

Occupational risk assessment identified a number of areas of concern. Risk of skin, eye and respiratory irritation exists when working in a confined space during cleaning of a storage tank for repacking. These risks may also occur during end use of pure limonene and/or products containing high concentrations of limonene, especially when used in confined spaces and in places with limited ventilation as well as by certain application modes such as spraying or where personal protective equipment (PPE) is not used.

The risk of health effects following repeated exposure is likely to be high during end use of limonene in confined spaces and in places with limited ventilation.

Significant environmental releases are expected to the aquatic compartment as a result of the use pattern in cleaners. However, as a result of dilution, degradation and evaporation these releases are not expected to result in adverse effects on aquatic organisms. No studies were identified on chronic effects, and therefore risks associated with chronic exposures of aquatic organisms to limonene in "polluted" waters cannot be determined.

Due to the high volatility of limonene the atmosphere is expected to be the major environmental sink for this chemical, where it is expected to rapidly undergo gas-phase reactions with photochemically produced hydroxyl radicals, ozone, and nitrate radicals. The oxidation of limonene may contribute to aerosol and photochemical smog formation. Ozonolysis of limonene may also lead to the formation of hydrogen peroxide and organic peroxides, which have various toxic effects on plant cells and may be part of the damage to forests observed in the last decades. However, limonene has not been identified as an air toxic in Australia and is not on the list of substances reported to the National Pollutant Inventory.

Potential hazards relevant to public health are skin irritancy and sensitisation from use of consumer products, varying with the concentration of limonene in the product and, for sensitisation, with its oxidation status.

In Australia transport and occupational use of limonene is controlled through a number of national standards and codes, corresponding State and Territory legislation and workplace controls. Limonene is not listed in the *Standard for the Uniform Scheduling of Drugs and Poisons.* It has not been identified as an air toxic in Australia and is not on the list of substances reported to the National Pollutant Inventory.

The major recommendations of the report focus on the autoxidation potential of limonene. As well as the current classification as a skin irritant, it is recommended that an additional risk phrase on skin sensitisation (R43 – May cause sensitisation by skin contact) be taken up in the National Occupational Health and Safety Commission (NOHSC) *List of Designated Hazardous Substances.* A range of measures is recommended to minimise autoxidation of limonene throughout its life cycle. Workplaces are asked to consider the hazards of spontaneous combustion, and of peroxides in distillation. Addition of oxidised limonene to the list of substances used in allergy testing is recommended.

As commercial limonene / dipentene is supplied under several Chemical Abstracts Service (CAS) numbers, suppliers are asked to ensure that hazard classification of these materials reflects the limonene content. Classification for aspiration hazard is also required under the NOHSC *Approved Criteria for Classifying Hazardous Substances* where the material meets viscosity and surface tension criteria. Other recommendations cover workplace controls, hazard communication and consideration of minor changes in transport coding.

Contents

LIST OF TABLES

LIST OF FIGURES

Acronyms and Abbreviations

ACS	Australian Customs Service
ADG Code	Australian Dangerous Goods Code
AICS	Australian Inventory of Chemical Substances
AIHA	American Industrial Hygiene Association
AS	Australian Standard
BCF	bioconcentration factor
BHA	butylated hydroxy anisole
BHT	butylated hydroxy toluene
BSA	body surface area
bw	bodyweight
CAS	Chemical Abstracts Service
CICAD	Concise International Chemical Assessment Document
conc.	concentration
CSIRO	Commonwealth Scientific Industrial Research Organisation
DHPA	dihydroperillic acid
DLOP	detection limit of the overall procedure
DOTARS	Department of Transport and Regional Services
EA	Environment Australia
EASE	estimation and assessment of substance exposure
EC	European Community, or European Commission
EC50	median effective concentration
EPG	Emergency Procedure Guides
EU	European Union
FFAANZ	Flavour and Fragrance Association of Australia and New Zealand
FID	flame ionisation detection
FMA	Fragrance Materials Association
FORS	Federal Office of Road Safety
GC	gas-chromatography
GC-MS	gas-chromatography/mass spectrometry
GRAS	generally recognised as safe
HPLC	high-performance liquid chromatography
HPV	high production volume

IARC	International Agency for Research on Cancer
IC(NA) Act	*Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth)
ICSC	International Chemical Safety Card
IFRA	International Fragrance Association
IPCS	International Programme on Chemical Safety
IRPTC	International Register of Potentially Toxic Chemicals
iv	intravenous
kg	kilogram
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol/water partition coefficient
kPa	kilopascal
L	litre
LC50	median lethal concentration
LD50	median lethal dose
LEV	local exhaust ventilation
LLNA	local lymph node assay
LOAEL	lowest-observed-adverse-effect level
LOEL	lowest-observed-effect level
m	metre
mg	milligram
$mg/cm^2/d$	milligram per centimetre square per day
mg/kg bw/d	milligram per kilogram bodyweight per day
MITI	Ministry of International Trade and Industry (Japan)
mL	millilitre
ML	megalitre
MOE	margin of exposure
mol	mole
MS	mass spectrometry
MSDS	material safety data sheet
NATO	North Atlantic Treaty Organization
NDPSC	National Drugs and Poisons Schedule Committee
ng	nanogram
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NIOH	National Institute of Occupational Health
NIOSH	National Institute of Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration

NOEL	no-observed-effect level
NOHSC	National Occupational Health and Safety Commission
NTP	National Toxicology Program
OECD	Organisation for Economic Cooperation and Development
OEL	occupational exposure limit
OSHA	Occupational Safety and Health Administration (USA)
PA	perillic acid
PEC	predicted environmental concentration
PNEC	predicted-no-effect concentration
POH	perilloyl alcohol
ppb	parts per billion
PPE	personal protective equipment
ppm	parts per million
RIFM	Research Institute for Fragrance Materials
RTECS	Registry of Toxic Effects of Chemical Substances (US)
s	second
SAR	structure-activity relationship
SCCNFP	Scientific Committee for Cosmetic Products, and Non-Food Products Intended for Consumers
SCBA	self contained breathing apparatus
SCID	Stored Chemicals Information Database
STEL	short-term exposure limit
STP	sewage treatment plant
SUSDP	Standard for the Uniform Scheduling of Drugs and Poisons
TGA	Therapeutic Goods Administration
TWA	time-weighted average (NOHSC)
TVOC	total volatile organic chemicals
UK HSE	United Kingdom Health and Safety Executive
US EPA	United States Environmental Protection Agency
v/v	volume per volume
VOC	volatile organic compound
WHO	World Health Organization
µg	microgram
µm	micromole

1. Introduction

1.1 Declaration

Limonene and its isomers were declared priority existing chemicals for full assessment by the Minister for Employment, Workplace Relations and Small Business under the *Industrial Chemicals (Notification and Assessment) Act 1989* (IC(NA) Act), as amended, by notice in the *Chemical Gazette* of 1 August 2000.

The reasons for the declaration were: the wide use of limonene in cleaning products, available for both industrial and consumer use; the chemicals are skin irritants in both humans and experimental animals and eye irritant in rabbits; and repeated exposure to limonene mainly affects the liver and kidneys. Use of limonene as a solvent will also result in direct exposure to the environment.

1.2 Objectives

The purpose of the assessment is to:

- identify the hazards of limonene to human health and the environment;
- identify use patterns and potential exposure in Australia; and
- characterise the risk of adverse effects resulting from exposure to workers, the general public, and the environment.

Use of the word 'limonene' in this report refers to the two isomers of limonene (*d*- and *l*-) and its racematic form *dl*-limonene, unless specified.

1.3 Source of information

Information for the assessment was obtained from various sources.

Industry

In accordance with the IC(NA) Act, manufacturers and importers of limonene who were manufacturing and/or importing and who wished to manufacture and import whilst limonene was a PEC were required to apply for assessment and supply information. Data supplied by applicants included:

- quantity of the chemicals and products containing the chemicals manufactured and imported;
- quantity of the chemicals formulated into products;
- uses of the chemicals and products containing the chemicals;
- methods used in handling, storing, manufacturing and disposal of the chemicals and products containing the chemicals;
- information on human and environmental exposure to the chemicals;
- Material Safety Data Sheet (MSDS) and labels; and

- a list of customers.

No unpublished data on health or environmental effects of limonene and its isomers were provided by applicants. Information for the assessment was also received from end users, formulators, and from a comprehensive literature search.

Literature review

The major sources of information on the health effects of limonene were the World Health Organisation (WHO) Concise International Chemical Assessment Document (CICAD) published under the International Programme on Chemical Safety (IPCS) covering limonene and its isomers (IPCS, 1998) and the International Agency for Research on Cancer (IARC) monographs on *d*-limonene (CAS 5989-27-5) (IARC, 1993; 1999). To enhance the efficiency of the National Industrial Chemical Notification and Assessment Scheme (NICNAS) assessment and provide transparency, the IPCS report (1998) was used as the basis for the hazard assessment, and the health effects sections of the CICAD were scanned into this report (Sections 9, 10, and 11). The primary studies from the IPCS report were not sighted and are indicated with an asterisk (*) in this report. The IPCS report included data available up to 1997. New information available since the IPCS report was identified from on-line searches of a number of publicly available databases. The IPCS report was also used as the basis of the environmental fate and toxicity review.

Surveys

Most of the applicants for the assessment on-sell the manufactured/imported limonene and were unable to provide any data on occupational exposure during use of the chemicals. NICNAS therefore conducted a telephone survey in August 2001 to investigate the use patterns, occupational exposure levels, control technologies and environmental exposure to limonene in Australia (NICNAS industry phone survey). Formulators and end users of limonene products participated in the survey by completing a questionnaire (see Appendix 1).

Site Visits

Information on mode of use and exposure was also obtained through a number of site visits.

1.4 Peer review

During all stages of preparation, the report has been subject to internal peer review by NICNAS, Environment Australia (EA) and the Therapeutic Goods Administration (TGA).

2. Background

Limonene occurs naturally in certain trees and bushes and is found in a large number of oils, including oils of lemongrass, citronella, palmarosa, cardamon and bergamot, siberian pine needle oil and several other essential oils including turpentine oils of various origins. It is a major constituent of oil of citrus rind, dill oil, oil of cumin, neroli, bergamot and caraway. Limonene is also one of the ingredients in the terpene fraction of tobacco smoke. Limonene and other monoterpenes are released in large amounts mainly to the atmosphere, from both biogenic and anthropogenic sources. The most widespread form is the *d*-limonene, followed by the racemic form *dl*-limonene (also called dipentene) and finally *l*-limonene.

d-Limonene was first recovered as a commercial product during the 1941-42 Florida (USA) citrus season, from the steam evaporator condensate in the production of citrus molasses. *d*-Limonene may be produced by steam distillation of citrus peels and pulp after alkali treatment, followed by fractional distillation. *d*-Limonene may also be extracted from citrus oils. *l*-Limonene may be produced by purification of isolated monoterpenes from certain pine needle oils, or synthetically from pinene by acid catalysis. *dl*-Limonene can be manufactured by mixing equal parts of *d*- and *l*-limonene, by thermal isomerization of α-pinene or as a by-product of the production of synthetic pine oils.

2.1 International perspective

Limonene is used globally as a flavour and fragrance additive in consumer products such as household cleaning products, perfumes and in beverages and food. It also has industrial applications, for example, as a solvent in degreasing metals prior to industrial painting and in paints, for cleaning in the electronic and printing industries, in manufacturing resins, as a wetting and dispersing agent and in insect control. *d*-Limonene and dipentene are listed on the Organisation for Economic Cooperation and Development (OECD) Representative List of High Production Volume (HPV) chemicals, i.e. production volume of 1000 tonnes or more in at least one OECD country.

International and national concerns about the health and environmental safety implications of limonene has resulted in a number of regulations and controls that have impacted on the use of limonene. Information on national regulations, guidelines and standards is available from the International Register of Potentially Toxic Chemicals (IRPTC) legal file at http://irptc.unep.ch/irptc/default.htm.

The flavour and fragrance industry is an important user of limonene. The International Fragrance Association (IFRA) represents the collective interests of the fragrance industry worldwide. The primary focus of IFRA is the worldwide development and advancement of the fragrance industry. The organisation seeks to preserve the self-regulatory practices of the international fragrance industry through the development and implementation of a Code of Practice and safety standards with the objective to protect the consumer and the environment.

The Code of Practice provides standards of good operating practice and product safety for the flavour and fragrance industry. It covers definitions of fragrance, basic standards of good manufacturing practice, use of fragrance materials, labelling and advertisement claims and international activities of the fragrance industry. Amendments and updates are issued on a regular basis.

Safety standards regarding use restrictions are based on safety assessments by the Panel of Experts of the Research Institute for Fragrance Materials (RIFM). RIFM is an independent scientific institute and their assessments are reviewed by the IFRA Scientific Committee. A restrictive list of fragrance ingredients (including limonene), containing recommendations for use that are considered essential in the interest of public health has been established and is maintained by RIFM through regular update. The recommendations are attached as Guidelines in Annex 2 of the Code of Practice. A RIFM review of limonene is currently in progress.

The voluntary standard for limonene recommends that *d*-, l- and *dl*-limonene and natural products containing substantial amounts of it should only be used when the level of peroxides is kept to the lowest practical level, for instance by adding antioxidants at the time of production. Such products should have a peroxide value of less than 20 mmol/L. This standard was made based on published literature mentioning sensitising properties when containing peroxides and oxidation products (International Fragrance Association, 2001).

In September 2001 the Scientific Committee for Cosmetic Products, and Non-food Products intended for Consumers (SCCNFP) of the European Union (EU) recommended that these standards be taken up in legislation for cosmetic products (European Union, 2001).

In America, limonenes are listed in the Code of Federal Regulations for Food and Drugs as generally recognised as safe (GRAS) chemicals.

2.2 Australian perspective

In Australia, consistent with overseas use, limonene is mainly used as a flavour and fragrance additive in consumer products in Australia. Concerns have been expressed by public interest organizations over its wide use and adverse health effects, including its sensitisation potential.

Dipentene (CAS No. 138-86-3) is listed in the National Occupational Health and Safety Commission's (NOHSC) *List of Designated Hazardous Substances* (NOHSC, 1999). Limonene is not listed in the *Standard for the Uniform Scheduling of Drugs and Poisons* (SUSDP) (NDPSC, 2001) and no Australian exposure standard has been established.

The Flavour and Fragrance Association of Australia and New Zealand (FFAANZ) is a member of IFRA and adopts the IFRA Code of Practices and Guidelines which are followed by most of the Australian flavour and fragrance manufacturers.

Most of the commonly used essential oils in Australia contain limonene. There are Australian Standards available for essential oils. In the early 1990's, the National Drugs and Poisons Schedule Committee (NDPSC) expressed concerns about the potential for poisonings from increasing use in the community of many essential oils. The Essential Oils Working Party was established in 1996 and identified those oils that warrant scheduling or prohibition or some form of safety packing and which can be used safely without any controls. Monographs for 38 essential oils were produced. Orange, lemon and lime oils where limonene is the major constituent are listed as safe oils. A final report developed by the working party (NDPSC, 1998) recommended that essential oils be considered individually for scheduling on the basis of documented scientific evidence of their toxicity hazard.

2.3 Assessments by other national or international bodies

Reviews of the health effects of limonene have been carried out by IARC (IARC, 1993; 1999), and by the Swedish National Institute of Occupational Health (NIOH) for the Nordic Expert Group (Karlberg & Lindell, 1993; Josefsson, 1993). The WHO published a report on limonene in 1998 (IPCS, 1998) based on the Swedish report. An International Chemical Safety Card (ICSC) was produced by IPCS in 1993 and reviewed in the IPCS report (1998). The ICSC was updated in 1997 (IPCS, 1997).

3. Applicants

Following the declaration of limonenes as priority existing chemicals, thirty-six companies and two interested parties applied for assessment of the chemicals. The applicants supplied information on the properties, manufacture and/or import quantities, MSDS and labels, uses of the chemicals. In accordance with the *Industrial Chemicals (Notification and Assessment) Act 1989*, NICNAS provided the applicants with a draft copy of the report for comments during the corrections/variation phase of the assessment. Data for the assessment were also provided by 11 notifiers, that is, companies that use limonene in Australia and are not applicants.

The applicants were as follows:

Amtrade International Pty Ltd
PO Box 6421
St Kilda Rd
Central Post Office
Melbourne
VIC 8008

Amway of Australia
PO Box 202
Castle Hill
NSW 1765

Asia Pacific Specialty Chemicals Limited
PO Box 232
Seven Hills
NSW 1730

Australian Council of Trade Unions
393 Swanston St
Melbourne VIC 3000

Australian Manufacturing Workers Union
3/440 Elizabeth St
Melbourne
VIC 3000

Haarmann & Reimer (Aust) Pty Ltd
PO Box 6005
Blacktown
NSW 2148

Holt Lloyd Australia Pty Ltd
PO Box 575
Castle Hill
NSW 2154

International Flavours & Fragrances (Aust) Pty Ltd
PO Box 695
Castle Hill
NSW 1765

Keith Harris & Co Ltd
7 Sefton Rd
Thornleigh
NSW 2120

Lightning Products Pty Ltd
PO Box 61
Williamstown
VIC 3016

Becot Pty Ltd (trade as Imtrade Commodities)
PO Box 395
Como
WA 6952

Bostik Findley Australia Pty Ltd.
PO Box 50
Thomastown
VIC 3074

Bronson and Jacobs Pty Ltd
Locked Bag 22
Homebush
NSW 2140

Campbell Brothers Ltd (Campbell Cleantec)
PO Box 490
Sumner Park BC
QLD 4074

Clariant (Australia) Pty Ltd
PO Box 23
Chadstone
VIC 3148

Colgate-Palmolive Pty Ltd
GPO Box 3964
Sydney
NSW 2001

Cosmark Pty Ltd
13/32 Campbell Ave
Dee Why
NSW 2099

Cussons Pty Ltd
282-300 Hammond Rd
Dandenong
VIC 3175

Loctite Australia Pty Ltd
PO Box 2622
Taren Point
NSW 2229

Merck Pty Ltd
207 Colchester Road
Kilsyth
VIC 3137

Natural Fractions Pty Ltd
PO Box 1234 Berri
SA 5343

Nowra Chemicals
5 Flinders Rd
Nowra
NSW 2541

Orica Australia Pty Ltd
PO Box 4311
Melbourne
VIC 3001

Peerless Emulsion Products Pty Ltd
PO Box 42
Fairfield
VIC 3078

Quantum Chemicals Pty Ltd
PO Box 4107
Dandenong South B.C
VIC 3164

Quest International Australia Pty Ltd
6 Britton St
Smithfield
NSW 2164

Dragoco Australia Pty Ltd
168 South Creek Rd
Dee Why West
NSW 2099

EKA Chemicals (Australia) Pty Ltd / Akzo Nobel
15 Conquest Way
Hallam
VIC 3803

Enzacor Pty Ltd. (trade as Fruitmark)
1/944-946 Glenhuntly Rd.
Caulfield South
VIC 3162

Fernz Specialty Chemicals
Locked bag 2008
Chester Hill
NSW 2162

Givaudan Australia Pty Ltd
9 Carolyn St
Silverwater
NSW 2128

G.R.Davis Pty Ltd
PO Box 123
Riverstone
NSW 2765

Redox Chemicals Pty Ltd
Locked Bag 60
Wetherill Park
NSW 2164

Selleys Pty Ltd
Locked Mail Bag 700
Milperra
NSW 1891

Semal Pty Ltd (trading as Consolidated Chemical Co.)
PO Box 999
Dandenong
VIC 3175

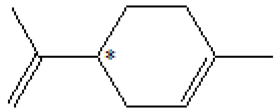
Septone Products Pty Ltd
44 Aquarium Avenue
Hemmant
QLD 4174

The Product Makers (Australia) Pty Ltd
50-60 Popes Rd
Keysborough
VIC 3173

Ungerer Aust Pty Ltd
PO Box 2143
Taren Point
NSW 2229

4. Chemical Identity and Composition

4.1 Chemical identity



Limonene occurs as the *d* and *l* isomers, and the racemic mixture *dl*-limonene known as dipentene. The chemical identities of these chemicals are listed in Table 4.1.

There are some changes in limonene isomer Chemical Abstracts Service (CAS) numbers as follows:

Chemical name	**Previous CAS number**	**Superseded with**
d-Limonene	7705-13-7	5989-27-5
	95327-98-3	
l-Limonene	7721-11-1	5989-54-8
Dipentene	7705-14-8	138-86-3

Commercial grades of limonene may also be imported under the following CAS numbers:

CAS number	**Chemical name#**
68647-72-3	Terpenes and terpenoids, orange oil
68917-57-7	Terpenes and terpenoids, mixed sour and sweet orange oil
68917-58-8	Terpenes and terpenoids, mixed grapefruit oil and shaddock oil
65996-99-8	Terpenes and terpenoids, turpentine oil, limonene fraction
68956-56-9	Hydrocarbons, terpene processing by product
8024-48-6	Orange, sweet, extract

#names listed in AICS.

Table 4.1 – Chemical identity of limonene

Chemical Identity	*d*-Limonene	*l*-Limonene	Dipentene
CAS No.:	5989-27-5	5989-54-8	138-86-3
IUPAC Name:	(R)-(+)-*para*-Mentha-1,8-diene	(S)-(-)-*para*-Mentha-1,8-diene	(±)-*para*-Mentha-1,8-diene
EINECS No.:	227-813-5	227-815-6	205-341-0
Molecular Formula:	$C_{10}H_{16}$	$C_{10}H_{16}$	$C_{10}H_{16}$
Structural Formula:	CH_3-C_6C_8-$C(CH_3)=CH_2$	CH_3-C_6C_8-$C(CH_3)=CH_2$	CH_3-C_6C_8-$C(CH_3)=CH_2$
Molecular Weight:	136.24	136.24	136.24
Synonyms:	FEMA 2633 (+)-4-Isopropenyl-1-methyl-1-cyclohexene *d*-(+)-Limonene (+)-(R)-Limonene *d*-p-Mentha-1,8-diene *p*-Mentha-1,8-diene (R)-1-methyl-4-(1-methylethenyl)-cyclohexene NCI-C55572	(-)-Limonene 1-methyl-4-(1-methylethenyl)-(s)-cyclohexene	Acintene DP Acintene DP dipentene Cajeputene Cinene Dipanol Inactive limonene Kautschin Limonene *dl*-limonene 1, 8(9)-*p*-Menthadiene 1-methyl-4-isopropenyl-1-cyclohexene Nesol -1,8-Terpodiene Unitene
Other Names:	Citrus terpenes Orange terpenes Menthadiene Terpene hydrocarbons 1,4-(8)-p-menthadiene Orange oil terpenes Cajeputene	None	None

5. Physical and Chemical Properties

5.1 Physical state

Limonene is a colourless liquid with a characteristic citrus odour at room temperature.

5.2 Physical properties

Physical properties of limonene are presented in Table 5.1. The values cited are from the IPCS report (1998) unless otherwise stated.

Table 5.1 - Physical properties

Property	*d*-Limonene	*l*-Limonene	Dipentene
Boiling point (°C)	175.5-176.0	175.5	176.0
Melting point (°C)	-74.35 -96.9*	-74.35	-95.9
Density (g/cm^3 at 20°C)	0.8411	0.8422	0.8402
Relative density (water = 1)	0.84#	0.84#	0.84#
Water solubility (mg/L at 25°C)	13.8	-	-
Vapour pressure	0.19 kPa at 20°C	0.19 kPa at 20°C	0.19 kPa at 20°C
Relative vapour density (air = 1)	4.7*	-	4.7 +
Henry's law constant (kPa m^3/mol at 25°C)	34.8	-	-
Partition coefficient (*Log Kow*)	4.23	-	4.83 4.23+
Lower flammable limit	0.7%#	0.7%#	0.7%#
Upper flammable limit	6.1%#	6.1%#	6.1%#
Autoignition temperature (°C)	237#	237#	237#
Flash point (°C)	48 (closed cup) #	48 (closed cup) #	45 (closed cup) # 48 (open cup)@

* IARC (1999), # Canadian Centre for Occupational Health and Safety (2001), + HSDB (accessed 2001), @ Gangolli (1999).

The vapour pressures of limonenes are not high, but the rate of vaporization of limonene in the environment may be high due to the low solubility in water and high value of Henry's law constant. Limonenes have been measured as volatile organic chemicals (VOCs).

Limonene is a flammable liquid. Explosive vapour/air mixtures may be formed at temperatures above 48°C (IPCS, 1998). Limonene liquid can float on water and may possibly travel to distant locations and/or spread fire. Mixtures of limonene vapour and air at concentrations in the flammable range may be ignited by a static spark as static charge may be accumulated by flow or agitation. (CCOHS, 2001).

Rags or other combustible material that have been wet or soaked with limonene may spontaneously combust. Peroxides formed by oxidation may be hazardous for explosion if they become highly concentrated through distillation.

5.3 Chemical properties

In the presence of light and air, autoxidation of limonene readily occurs to give a variety of oxygenated monocyclic terpenes. The oxidation products of *d*-limonene identified (Karlberg & Dooms-Goossens, 1997) include:

- (+)-*cis*-limonene-1,2-oxide (1,2-epoxy-*p*-mentha-8-ene);
- (+)-*trans*-limonene-1,2-oxide (1,2-epoxy-*p*-mentha-8-ene);
- *l*-carvone ((R)-(-)-6,8-*p*-mentha-diene-2-one);
- (-)-*cis*-carveol (*cis*-2-hydroxy-*p*-mentha-6,8-diene);
- (-)-*trans*-carveol (*trans*-2-hydroxy-*p*-mentha-6,8-diene);
- *cis*-limonene-2-hydroperoxide (*cis*-2-hydroperoxy-*p*-mentha-6,8-diene); and
- *trans*-limonene-2-hydroperoxide (*trans*-2-hydroperoxy-*p*-mentha-6,8-diene).

The primary oxidation products are hydroperoxides, which are unstable and readily degrade to other secondary oxidation products such as carvone, if further subjected to daylight and air (Nilsson et al., 1999). If oxidation process continues, polymers are created and the liquid will become viscous (Karlberg and Dooms-Goossens, 1997).

When limonene is heated to decomposition it emits carbon monoxide and carbon dioxide (CCOHS, 2001).

5.4 Impurities and additives

Impurities in *d*-limonene are mainly other monoterpenes, such as:

- myrcene (7-methyl-3-methylene-1,6-octadiene),
- α-pinene (2,6,6-trimethyl-bicyclo[3.1.1]-hept-2-ene),
- β-pinene (6,6-dimethyl-2metylene-bi-cyclo[3.1.1]heptane),
- sabinene (2-metyl-5-(1-metyl-ethyl)-bicyclo[3.1.0]hexan-2-ol),
- Δ3-carene ((1S-cis)-3,7,7-trimetyl-bicyclo[4.1.0]hept-2-ene).

Information from industry indicates that the *dl*-limonene content of commercial grade dipentene is 10% to 70%. The name dipentene in scientific literature refers to racemic limonene, but in industry may refer to any mixture of terpene hydrocarbons, usually *p*-menthadienes [Zinkel, 1989]. Some imported dipentene is obtained from the manufacture of synthetic pine oil. However, other manufacturing methods may also be used.

Some limonene grades are supplied with antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and preservatives, for example, sodium erythorbate and sodium benzoate.

5.5 Conversion factors

The conversion factors for limonene are:

1 ppm = 5.56 mg/m^3

1 mg/m^3 = 0.177 ppm

6. Methods of Detection and Analysis

6.1 Identification

The isomers of limonene are usually determined by gas chromatography using flame ionisation detection (GC-FID) or mass spectrometry (GC-MS).

As limonene is easily oxidised in air, it is also important to analyse the oxidation products. Hydroperoxides of *d*-limonene can be characterised by GC if the sample is injected on-column (IPCS, 1998). GC-MS with chemical ionisation in negative ion mode was shown to be a successful method for the identification and determination of the molecular weight of chemically unstable limonene hydroperoxides (Nilsson et al., 1996). A high performance liquid chromatography (HPLC) method for isolation of individual compounds in autoxidised *d*-limonene has been developed by using 2 different stationary phases in normal phases mode (Nilsson et al., 1996). The method can be used for the isolation of individual contact allergens in sensitisation experiment vehicles, such as petroleum and olive oil.

A sensitive GC-MS method using stable-isotopically labelled internal standards has been reported (Zhang et al., 1999), for the quantitation of a metabolite of *d*-limonene, perillyl alcohol (POH), and its metabolites, perillic acid (PA) and *cis*- and *trans*-dihydroperillic acid (DHPA). The products were separated on a capillary column and analysed by an ion-trap GC-MS using NH_3 chemical ionisation. The quantitation limits for POH, PA, *cis*- and *trans*-DPHA were < 10 ng/mL using 1-2 ml plasma.

6.2 Atmospheric monitoring methods

The determination of limonene in ambient air can be achieved by either passive or active means. Passive sampling involves adsorption onto activated charcoal with desorption by carbon disulfide followed by capillary GC. Active methods include adsorption onto Tenax (a porous polymer) using an air flow rate of 100 to 300 ml/min (up to 3 L of ozone free air can be sampled on 200 mg Tenax) (Janson & Kristensson, 1991) or multisorbent sampling tubes (Chan et al., 1990) and separated by capillary GC. Thermal desorption at 250 ^{o}C for 5 minutes is followed by GC-MS. The limit of detection was not stated.

National Institute of Occupational Safety and Health (NIOSH) method 1552 and Occupational Safety and Health Administration (OSHA, USA) method PV2036, for the determination of occupational airborne limonene entails the passage of 10 L of sample volume over activated charcoal at sampling rates of 10 to 200 mL/min for long-term sampling (Eller & Cassinelli, 1994; OSHA, 1994) and 250 mL/min for short-term sampling (Josefsson, 1993). Desorption is achieved with carbon disulfide followed by GC-FID. The detection limit is 0.4 µg/sample (4 µg/m^3). The detection limit of the overall procedure (DLOP) is 1.3 µg/sample (0.13 µg/m^3). The DLOP is defined as the concentration of analyte that gives a response that is significantly different from the background response (OSHA, 1994).

6.3 Biological monitoring methods

The presence of *d*-limonene in biological samples including body fluids, blood and tissues can be detected by a head-space technique (Falk-Filipsson et al., 1993). The samples (blood, urine) are transferred to gas-tight head-space vials (22.4 ml) and capped immediately with Teflon-lined membranes. The head-space air of the samples is analysed automatically on a GC equipped with a head-space auto-sampler, a FID, and a polar column. The concentration of *d*-limonene in the samples is determined by comparison with individual standard curves prepared in the same concentration range by adding *d*-limonene to the samples taken before the exposure. The limit of detection in blood is 1.4 μg/L.

6.4 Monitoring methods for natural products

d-Limonene has been measured in a range of natural products, such as orange juice, by GC and head-space analysis and in packaging materials by thermal desorption (IARC, 1993).

Oils separated by distillation from orange, tangerine and grapefruit juices contain at least 98% *d*-limonene. The *d*-limonene content of such oils has been determined by co-distillation with isopropanol, acidification and titration with potassium bromide-potassium bromate solution. The distribution of optical isomers of limonene has been determined in various essential oils using multidimensional GC, by coupling chiral and nonchiral columns (IARC, 1993).

7. Use, Manufacture and Importation

7.1 Manufacture

d-Limonene is manufactured in Australia from orange oils by extraction through vacuum distillation. Another major manufacture method, which is not reported in Australia, is as a by-product of orange oil by washing of cold pressed oil. This method is reported to be the source of some imported materials. Both imported and locally sourced orange oils are used for manufacturing *d*-limonene. Manufacturing plants identified during this assessment are located at Riverstone, NSW, Renmark, SA and Williamstown, VIC. Approximately 100 to 130 tonnes of *d*-limonene is produced per year. Approximately 15 tonnes a year of *d*-limonene was also manufactured at Thornleigh, NSW until 1999. It is likely that there may be more limonene producers in Australia that have not been identified by NICNAS. There is no report of dipentene and *l*-limonene manufacture in Australia.

Orange oils extracted from orange peel in Australia (quantity unknown) are an additional source of limonene.

7.2 Natural occurrence

Like other monoterpenes, limonene occurs naturally in certain trees and bushes and is found in the peel of citrus fruits, dill, caraway, fennel, celery and in turpentine. High levels of limonene are present in some oils, for example, orange (> 90%), grape fruits (90%), lemon (70%) and celery (60%). *d*-Limonene is the main constituent in oil of citrus fruits and also in many other essential oils such as oils of dill, caraway and fennel. *l*-Limonene is found mainly in pine-needle oils and turpentine but also in, for example, spearmint and peppermint. Dipentene is the racemic mixture of *d*- and *l*-limonene.

Monoterpenes are released in significant amounts mainly to the atmosphere. No information on the levels of monoterpenes in air in Australia is available. According to the IPCS report (1998), the levels of monoterpenes in air vary largely but typical concentrations in conifer forests are 1-10 $\mu g/m^3$. It was reported that the mean emission rates of limonene from different plant species in the Central Valley of California ranged from 0.4 to 2.5 mg/g dry leaf weight per hour (IPCS, 1998). Biogenic emissions are in the order of those from anthropogenic sources. Global annual emissions of biogenic monoterpenes range from 147 to 827 million tonnes.

7.3 Importation

Importers of limonene and products containing limonene were requested in August 2000 to provide information on import quantities over a 2-year period from August 1998 to August 2000 and predicted quantities for the year 2000-2001. Approximately 3000 tonnes of *d*-limonene and dipentene were reported to be imported in the 2-year period from a number of countries. Most of *d*-limonene is imported in 200 L steel drums or epon lined steel drums and some in 173 kg nett, new mild steel drums. Dipentene is imported either in 175 kg metal drums or 200 L epon lined steel drums. No *l*-limonene (CAS 5989-54-8) is imported into Australia.

Information on orange oil was not specifically requested from industry during the assessment. Information obtained from the Australian Customs Service (ACS) indicates that the quantities of orange oil imported in the two-year period (1998-2000) were about 1000 tonnes. Quantities of orange oils produced in Australia are not known. Orange oils may contain *d*-limonene as high as 96%. Large amounts of orange oil may be used in food applications.

Products containing limonene, primarily intermediate fragrance blends, and some end-use consumer products, are also imported into Australia and the concentrations of limonene in products vary widely, from very low to 90%. The total reported import quantities of limonene (*d*-, *l*-, *dl*-) contained in products in the 2-year period were approximately 120 tonnes. Most of the fragrance blend products are further incorporated into end products. Some final end use products containing limonene are also imported and sold directly to the general public without reformulation or repacking. Few imports of final end-use products for occupational use were reported.

The reported import quantities for *d*-, *l*- and *dl*-limonenes are listed in Table 7.1.

Table 7.1 – Reported import quantities of limonenes in financial years 1998-2001

		1998-1999 (tonnes)	1999-2000 (tonnes)	2000-2001 (tonnes)	% limonene in product
***d*-Limonene**	Raw material	1366	1435	1374	
	In products	24	23	16	< 0.2-70
Dipentene	Raw material*	70	67	55	
	In products#	0.14	0.07	0.09	0.001-30
***l*-Limonene**	Raw material	0	0	0	
	In products	3.9	3.9	unknown	< 1-90
Limonene	Raw material	0.05	0.5	0	
(isomer unspecified)	In products	31	31	31	Very low (ppm-90)
Total Limonene raw material		**1436**	**1502**	**1429**	
Total Limonene in products		**59**	**58**	**unknown**	
Grand Total		**1495**	**1560**		

*The quantity shown here is the total amount of commercial grade of dipentene imported. The purity of dipentene was reported to be 10% to 70% (see Section 5). In addition, approximately 200 tonnes of other grades of pine oil are imported into Australia every year. These contain lower levels of dipentene, for example, < 1%, 2-5%, 5-10% and were not included in Table 7.1.

#The purity of dipentene in the imported products is not known.

Information on importation quantities of limonene in financial years 1998/1999, 1999/2000 and part of year 2000/2001 was also obtained from the ACS and is shown in Table 7.2. Chemicals are imported into Australia under tariff codes according to the Australian Customs tariff. Most imports of limonene occur under tariff code 2902.19.00 08 (‘other cyclic hydrocarbons’). They can also occur under other tariff codes, specifically those covering crude dipentene, and terpenes obtained from essential oils. As these codes cover a number of other chemicals apart from limonene, data in Table 7.2 include only those shipments under these codes that were identified specifically as limonene in textual descriptions provided to the ACS.

Table 7.2 - Yearly total imports of *d*-, *l*- and *dl*- limonene in financial years 1998-2001 identified under the ACS tariff code

Year	**1998-1999**	**1999-2000**	**2000-2001 (11 months)**
Tonnes imported	526.4	381.4	655

There is a discrepancy between the total amounts of limonene reported as imported (Table 7.1) and the amounts recorded by the ACS (Table 7.2). It is possible that some limonene shipments were not explicitly identified as such in the shipping descriptions and were therefore not included in Table 7.2 (as explained above). In addition, analysis of the data has shown that it is likely that a large amount of limonene imports were shipped under a tariff code covering essential oils and were not represented in Table 7.2. Another factor that may contribute to the discrepancy between the figures is the slight difference in the periods reported by industry and that captured by the customs data. Industry reported for yearly periods starting in August 1998, whereas the customs data covered financial years starting July 1998.

7.4 Repacking, reselling and formulation of products in Australia

Limonene in Australia is either used by the manufacturer and/or importer and/or supplied to formulators to produce intermediate or end products. A similar pattern exists for imported products containing limonene. The distribution chains vary as repacking and reselling may occur as intermediate steps. This is seen with both limonene and products containing limonene, either locally manufactured or imported. The distribution chains of manufactured and/or imported limonene are shown in Figure 1. Figure 2 shows the distribution chains of imported products containing limonene.

7.5 Uses

Information on uses of limonene and products containing limonene was provided by industry and also obtained by the NICNAS phone survey. The survey attempted to reach users of limonene through the distribution chain. The formulators and end users were selected randomly, covering a wide range of industry sectors, from customer lists provided by importers and manufacturers. However, the profile of users contacted during the survey might not be representative as only a small proportion of formulators and users of limonene were contacted.

7.5.1 Use of limonene

d-Limonene and dipentene may be formulated into intermediate or end products, or used as they are.

Figure 1. Distribution chain of manufactured/imported limonene

x= Repacking is possible

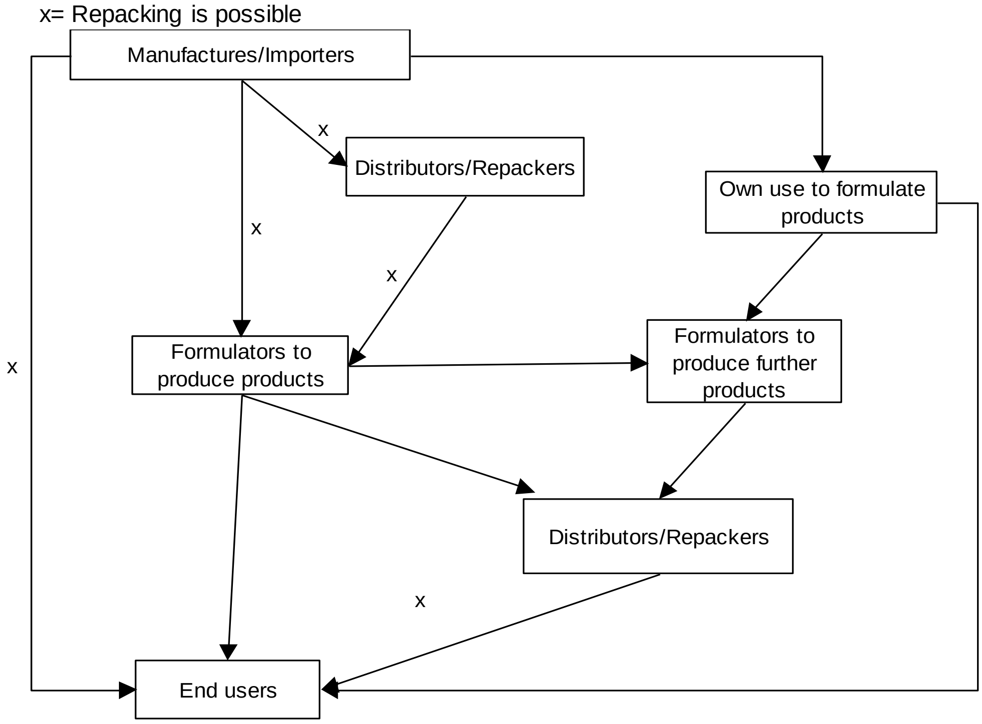
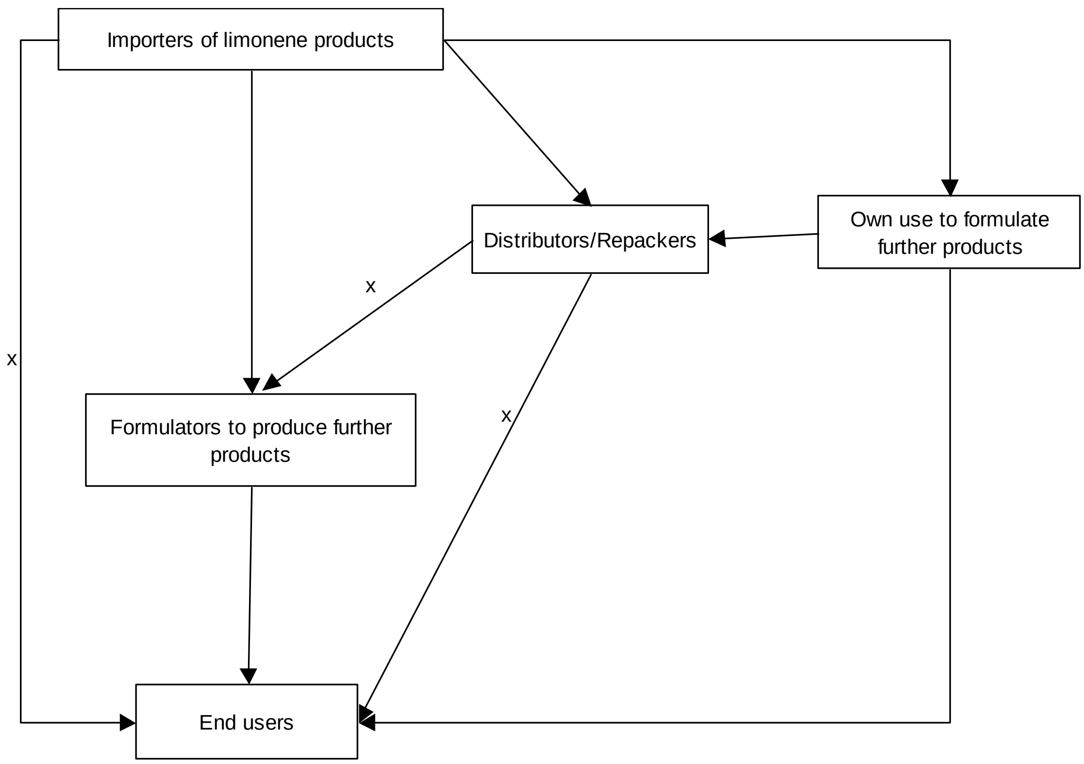


Figure 2. Distribution chain of imported limonene products



x= Repacking is possible

d-Limonene and dipentene are mainly used in the formulation of a range of fragrance or flavour blends that are used to further formulate end products for industry and consumer use or as food additives. Some of the *d*-limonene and dipentene are used to formulate end products directly. Information provided by importers indicates that *d*-Limonene and dipentene are both used in the formulation of the following products:

- as replacement for toxic chlorinated solvents or as solvent carriers;
- flavour and fragrance additives;
- essential oils;
- wetting and dispersing agents;
- resins and specialty adhesives;
- cleaners for floor, printing inks and press, spot and stain, metal, marine vessels electronics, circuit board, and concrete;
- hand cleaners;
- personal or air deodorants;
- masking agents for rubbish tips.

d-Limonene is also used as a heat transfer medium; release agent; in asphalt grading; and in graffiti, adhesive, tar and asphalt removers and in the production of degreaser/grease trap maintainer to help dissolve grease and keep foul odours down in grease traps.

It was reported that there is a decreasing trend in formulating perfume products using limonene. The major reason is claimed to be the efficient automated process used overseas which is too high cost for the small market in Australia.

Some *d*-Limonene and dipentene are used directly for the following industrial purposes:

- degreasing agent as liquid or aerosol;
- glass mould cleaning;
- polystyrene recycling (stripping) and rubber stripping;
- air craft part cleaning;
- as an adhesive agent in rubber treatment;
- wax remover from laboratory slides prior to mounting and for removing hard depilatory wax from floor and instruments in salons;
- cleaning tanks with viscous residues;
- microbiological and gemstone testing.

Uses of high dipentene grade of pine oil in Australia are reported to be as a solvent for paints, wool, cleaning compounds, disinfectants and polishing solutions.

A small quantity of limonene (type of limonene not specified) is used in laboratories for testing for quality control and as a general laboratory reagent.

Availability of limonene to the general public was not reported.

7.5.2 Use of industrial products containing limonene

Both imported and formulated products containing limonene are used either directly or further formulated into products for end use. They have a wide range of applications in Australia.

d-Limonene

Hand cleaners used in workshops

The largest use of products containing *d*-limonene in industry is as heavy-duty hand cleaners. They are used widely in industrial workshops in Australia. Information obtained from suppliers and the NICNAS industry phone survey indicates that the concentration of *d*-limonene ranges from 2% to 35%. The package sizes and types vary largely between different manufacturers, but in most cases, the package sizes range from 70 g to 200 kg and package types can be soft tube with a screw cap, plastic bottles with a cap or pump dispenser, steel tin with a cap or lid, and pumps and wall bracket. The quantities and number of formulations obtained during the survey are not representative due to the small survey size.

Industrial cleaning

Industrial cleaning products containing *d*-limonene have a wide range of applications and concentration of *d*-limonene varies largely. Information on uses, concentrations of *d*-limonene and package sizes is listed in the Table 7.3.

Table7.3 - Use of products containing *d*-limonene in industrial cleaning

Uses	% *d*-limonene	Package size
General degreasing and general purpose cleaning of equipment, floor and oven in various industry sectors such as mining, automotive, hospitality, marine and aircraft industry	< 1 - > 60	Varies
Cleaning of printing press to wash out printing inks	5 - 35	20 L, 25 L and 200 L steel drums
Industrial metal cleaning	< 1	Varies
Industrial laundry pre-spotter and detergent	2 to 4	Varies

Remover and stripper

Products containing *d*-limonene are used as removers and/or strippers for industrial purposes. The applications, concentration of *d*-limonene and package size are listed in Table 7.4.

Fire extinguishant blends

Use of 2 fire extinguishant products, NAF S-III and NAF P-III containing about 4% limonene, was not reported by industry during the assessment, but was reported in the PEC report on HCFC-123 (NICNAS, 1999). Approximately 40 tonnes of each product were imported into Australia in 1996 to replace halon extinguishants. NAF S-III has been used as a 'total flooding agent' in fixed systems and NAF P-III as a 'streaming agent' in portable extinguishers. Both blends are particularly suitable for fires involving computer or electrical equipment. The NICNAS report (1999) stated that the importation of these 2 products is expected to decline as HCFCs are gradually being phased out.

Table 7.4 - Use of products containing *d*-limonene as remover and/or stripper

Uses	% *d*-limonene	Package size
Wax stripper for stripping floor polish in hotels/motels	< 1 – 3	Unknown
Ink remover	95	Unknown
Graffiti remover	10 - 30	4, 10, 20 L
Sealant and adhesive stripper	2 - 30	Unknown
Paint stripping	19	500 mL, 1 L, 4 L

Miscellaneous uses

Other reported industrial uses of products containing *d*-limonene are listed in Table 7.5.

Table 7.5 - Miscellaneous uses of *d*-limonene products

Uses	% *d*-limonene	Package size
Car detailing		
• removing gum, grease, tar spots from upholstery	78	500 mL spray pack,
• tyre gloss	< 1	5, 20, 200 L containers
• vehicle washing product	1-10	
• automotive paints	20	
Lubricant used for lubricating wire rope	20	Unknown
Masking agent for industrial purposes	0.125 – 0.3	Unknown
Paint formulation as a preparation agent	19	Unknown
Paper softener	0.5	15 kg pail, 900 kg "Schutz" container
Textile industry to scour dirt and grease out of textiles/cloth on an industrial scale	< 20	Unknown
Timber finishing as a timber furniture and floor polish	1.8 – 3.5	Unknown

Dipentene

Dipentene is used in formulating a number of products with similar uses as *d*-limonene products.

***l*-Limonene**

It is reported that fragrance blends containing < 1% to 90% *l*-limonene are used in formulation of industrial cleaners. The concentrations of *l*-limonene in the final products are not known, however, it was reported to be at very low levels.

In addition to the uses described above, importers and manufacturers indicated the other uses shown in Table 7.6.

Table 7.6 - Other uses of limonene products reported by importers and manufacturers

Limonene isomer	Reported Uses
***d*-limonene**	trials in natural dyeing of polyester in rubber industry in leaching
Limonene (Type of limonene non specified)	first aid products

7.5.3 Use of consumer products containing limonene

Flavouring and fragrance agents used in consumer products

Both imported and formulated perfumes and fragrances containing limonene are further diluted when used in consumer products. For example, typical use levels of fragrances in household products such as cleaners, soaps etc. are 0.5 – 2% and for cosmetic products such as lotions and colognes are 0.5 – 20%. Flavouring agents containing limonene are used in the food and pharmaceutical industries with the levels of limonene in food and medicine reported as “very low”.

The information on uses, concentrations of limonene and package sizes for some imported consumer products was provided by one of the importers and shown in Table 7.7. These consumer products containing limonene are imported and sold directly to the general public without reformulation and repacking.

l-Limonene is imported as an ingredient in fragrance and used in air fresheners, household and personal care, hair care, fabric care and laundry products. The range of *l*-limonene content in the imported perfume blend is < 1% to 90%. However, the level of *l*-limonene in final products is usually low.

Essential oils

Limonene is present in essential oils used in Australia and there is increased use in the community of a wide range of these oils. The Essential Oil Working Party report (NDPSC, 1998) indicates that 22 out of 38 essential oils assessed contain limonene at levels ranging from 0.1% to 96%. The use of essential oils is widespread and these are available for a variety of topical applications either in the pure form or compounded with other ingredients. Essential oils are used in:

- aromatherapy, in which the oils are applied for local effects and possible systemic effects through inhalation and dermal absorption;
- as environmental fragrances, where the oils are used in burners or aerosol diffusers;

- for therapeutic purposes, in which the oil can be inhaled by various means for respiratory ailments, used in baths or in a compress;
- as a repellent, where the oil is usually present in a finished product although the pure oils can be available for this purpose;
- for use in foods as flavouring agents. The pure essential oils used for this purpose can be incorporated into flavouring agents at a lower concentration or mixed directly into food preparations such as icing and chocolates; and
- for household cleaning purposes such as removing grease spots from fabrics and in specially formulated "wool-washes".

Table 7.7 - Uses of imported consumer products containing limonene

End products	% Limonene	Use	Package size
***d*-Limonene**			
Deodorant	0.125		50 g
Perfume spray	0.10 - 0.18		75 mL
Shaving foam	0.05		250 g
Body lotion	0.04		200 mL
Liquid detergent	0.04		1 L
Shower gel	0.015		200 mL
Liquid organic cleaner	0.005-0.0075	Is versatile for multi-purpose cleaning	1 L, 4 L
Dishwashing liquid	0.00225		1 L, 4 L
Towelettes	0.000075	For convenient spot cleaning	88.5 g
Dipentene			
Furniture polish	0.05	Cleaning, polishing and protecting furniture and for adding a glow to finished wood and leather	350 g
Leather and vinyl cleaner	0.02	Deep cleaning and conditioning all colours of leather and vinyl with abrasives	500 mL
Laundry compound	0.075	Concentrated detergent for difficult wash conditions	1kg, 3 kg
Shaving foam	0.005	For a smooth and close shave	250 g
Foam carpet cleaner	0.001	Spot clean for carpets and upholstery	600 g

Table 7.8 lists concentrations of essential oils in consumer products (NDPSC, 1998).

Table 7.8 - Concentrations of essential oils in consumer products

Products	% of Essential Oils
Cosmetics and toiletries	1-5
Personal fragrances	5-20
Cleaning products	1-10
Deodorants	1-10
Food	Very low
Compounded essential oil products	
Massage oils	4-5
Products sold in supermarkets	< 2

Other reported consumer uses

The other consumer uses reported to NICNAS are:

Product	% of Limonene
Timber furniture and floor polish	70
Bug and tar remover as a solvent	22
Paint stripper	19
Shoe finish products	15-16
DIY cleaning products for boat canopies	5
Timber finish for the DIY timber finish market	1.5
Tyre shine aerosols	0.5
Dental care products used for cleaning false teeth	< 0.125
Carpet and upholstery cleaning products in aerosol	0.05
Car wash products	2
General cleaners	2
Pesticides products as insect repellents (as an attractant) and insecticides	Unknown

7.6 Exports

Both limonene and products containing limonene are exported to more than 30 countries in the world. The quantities exported are not known.

8. Exposure

8.1 Environmental exposure

8.1.1 Environmental release

Limonene is both manufactured within and imported into Australia in large quantities. Imports include the raw material and formulated products. Releases may be expected through the manufacture, formulation processes and end use of formulated products.

It can be clearly seen from Section 7 that this chemical has a diverse range of applications. These uses have considerably different release rates to the environmental compartments. For example, virtually all the limonene present in hand cleaners and laundry detergents would be expected to be discharged to sewers, whereas that present in furniture polish and paint formulations would have minimal release to sewers.

8.1.2 Environmental fate

No data on environmental fate data have been provided. The following fate results have mainly been obtained from the Concise International Chemical Assessment Document (IPCS, 1998) on limonene. The test reports, and figures are stated as reported and have not been sighted.

The chemical and physical properties of limonene indicate that the substance will be distributed mainly to air. Level 1 McKay modelling indicates that 97.8% will partition to air, 1.9% to soil, 0.25% to water and 0.04% to sediment.

Aquatic fate

In the aquatic environment, limonene is expected to adsorb to sediment and suspended organic matter and to rapidly volatilise to the atmosphere, based on its physical/chemical properties. The estimated half-life for volatilisation of limonene from a model river (1 m deep, flow 1 m/s and wind speed 3 m/s) is 3.4 h (IPCS, 1998).

Atmospheric fate

In the atmosphere, limonene is expected to rapidly undergo gas-phase reactions with photochemically produced hydroxyl radicals, ozone, and nitrate radicals. Calculated lifetimes for the reaction of *d*-limonene with photochemically produced hydroxyl radicals range from 0.3 to 2 h, based on experimentally determined rate constants. The corresponding lifetimes for the reaction with ozone ranged between 0.2 to 2.6 h. Based on experimentally determined rate constants, calculated lifetimes for the night-time reaction of *d*-limonene with nitrate radicals range from 0.9 to 9 min. The daytime atmospheric lifetime of *d*-limonene has been estimated to range from 12 to 48 min, depending upon the local hydroxyl radical and ozone concentrations (IPCS, 1998).

Products formed from the hydroxyl radical reaction with limonene are 4-acetyl-1-methylcyclohexene, a keto-aldehyde, formaldehyde, 3-oxobutanal, glyoxal, and a

C10 dicarbonyl. The same carbonyls, along with formic acid and C8 and C9 carboxylic acids, may also form in reactions with ozone. Ozonolysis of limonene may also result in bis(hydroxyrnethyl)peroxide, a precursor to hydroxymethyl hydroperoxide, and hydrogen peroxide. Hydroxymethyl hydroperoxide, bis(hydroxymethyl)peroxide, and hydrogen peroxide have various toxic effects on plant cells and enzymes. The reaction of *d*-limonene with ozone in the dark results in the formation of 4-acetyl-1-methylcyclohexene and formaldehyde. Reactions with oxides of nitrogen produce aerosol formation as well as lower molecular weight products, such as formaldehyde, acetaldehyde, formic acid, acetone, and peroxyacetyl nitrate (IPCS, 1998).

Terpenes such as limonene contribute to aerosol and photochemical smog formation. Emissions of biogenic hydrocarbons such as limonene and other terpenes to the atmosphere may either decrease ozone concentrations when oxides of nitrogen concentrations are low or, if emissions take place in polluted air (i.e. containing high oxides of nitrogen levels), lead to an increase in ozone concentrations (IPCS, 1998).

Terrestrial fate

When released to ground, limonene is expected to have low to very low mobility in soil, based on its physical/chemical properties. The soil adsorption coefficient (K_{OC}), calculated on the basis of the solubility (13.8 mg/L at 25°C) and the log octanol/water partition coefficient (4.23), ranges from 1030 to 4780 (IPCS, 1998). The Henry's law constant indicates that limonene will rapidly volatilise from both dry and moist soil; however, its strong adsorption to soil may slow this process (IPCS, 1998).

Biodegradation and bioaccumulation

Limonene does not have functional groups for hydrolysis, and its cyclohexene ring and ethylene group are known to be resistant to hydrolysis (US EPA, 1994). Therefore, hydrolysis of limonene is not expected, in terrestrial or in aquatic environments. The hydrolytic half-life of *d*-limonene has been estimated to be > 1000 d. Biotic degradation of limonene has been shown with some species of micro-organisms, such as *Penicillium digitatum, Corynespora cassiicola, Diplodia gossypina* (IPCS 1998), and a soil strain of *Pseudomonas* sp. (PL strain). As these studies were not designed to determine the biodegradability of limonene, the results provided only indications of possible biodegradation. However, limonene was readily biodegradable (41-98% degradation by biochemical oxygen demand in 14 d) under aerobic conditions in a standard test (OECD 301 C "Modified MITI Test (1)"; OECD, 1981a; MITI, 1992). Also, in a test simulating aerobic sewage treatment (OECD 303 A "Simulation Test - Aerobic Sewage Treatment: Coupled Units Test"; OECD, 1981b), limonene disappeared almost completely (> 93.8%) during 14 d of incubation (IPCS, 1998). However, this test was not suitable for such a volatile substance as limonene. The disappearance of limonene was likely due in part to volatilisation, but it could not be determined to what extent the removal was due to biodegradation and sorption compared with volatilisation (IPCS, 1998).

Biodegradation has also been assessed under anaerobic conditions. In a test on methanogenic degradation (batch bioassay inoculated with granular sludge, 30 °C), there was no indication of any metabolism of limonene, possibly because of toxicity to the micro-organisms (IPCS, 1998). Complex chlorinated terpenes, similar to toxaphene (a persistent, mobile, and toxic insecticide, with global distribution) and its degradation products, were produced by photoinitiated reactions in an aqueous system initially containing limonene and other monoterpenes, simulating pulp bleaching conditions (IPCS, 1998).

The bioconcentration factor, calculated on the basis of water solubility and the log octanol/water partition coefficient, is 246-262 (IPCS, 1998), suggesting that limonene may bioaccumulate in fish and other aquatic organisms.

8.1.3 Predicted environmental concentrations in the aquatic compartment

The predicted environmental concentrations (PECs) of limonene in water have been calculated according to the methods in the Technical Guidance Document (European Commission, 1996). Releases have been outlined in Table 8.1. The quantities are based on the information provided in the NICNAS surveys and company submissions. A large number of these surveys and submissions did not contain use volumes. In addition, the total volumes determined represents only around 1/10th of the total limonene imported or produced in Australia each year. Hence, the calculated figures are likely to represent an underestimate of the actual releases.

Table 8.1 - Releases of limonenes to water

Product Type	Cleaners		Solvents		Other	
Isomer	*d*-limonene	dipentene	*d*-limonene	dipentene	*d*-limonene	dipentene
Volume Used (tonnes/Year)	309	23.7	16.8	-	122.3	14
Release Rate	100%	100%	1%	-	1%	1%
Release to Sewer (tonnes/Year)	309	23.7	0.17 [a]	-	1.2[a]	0.14[a]

[a]Assumes 1% release through formulation.

PEC

The PEC for water can be calculated using the daily discharge, volume of waste water removal rate in the sewage treatment plants (STPs) and dilution rate in receiving waters according to the following equation. The daily discharge to water has been estimated following assumptions outlined in Section 8.1.1.

$$PEC_{water} = \frac{PEC_{effluent}}{D}$$

Where:

PEC_{water} = predicted environmental concentration in receiving water (mg/L).

$PEC_{effluent}$ = concentration of the chemical leaving the STP (mg/L)

D = Dilution factor.

$$PEC_{effluent} = PEC_{sewer} \cdot \left(\frac{100 - P}{100}\right)$$

Where:

P	=	percentage removal in the sewage treatment plant.
PEC_{sewer}	=	concentration of the chemical entering the STP (mg/L)
PEC_{sewer}	=	$\frac{W}{Q}$

Where:

W	=	emission rate (kg/d).
Q	=	volume of wastewater (ML/d).

Values:

D	=	10 (high dilution eg ocean outfall) or 2 (low dilution, river/creek discharge)
Q	=	4940 ML (Assumes 260 L per person per day released to sewers Australia wide for a population of 19 million, based on Australian Bureau of Statistics figures for water usage (1,707 GL for 1996/97; ABS 2002) and population (18 million in 1996; ABS 2000)
P	=	97%

Assuming d-limonene is classified as readily biodegradable, with a LogH 4.54, and Log Pow 4.21, the SIMPLETREAT model estimates that 55% will partition to air, 3% will partition to water, 32% will partition to sludge, and 10% will be degraded over the retention period in the STP (European Commission, 1996). This gives total removal in the STP of 97%.

These equations have been used to calculate PEC for the surface water compartment based on release of limonene when processed from a raw material in Australia, either for formulation or end use of limonene products. The PECs calculated are given in Table 8.2.

The $PEC_{effluent}$ is equivalent to the $PEC_{(surface\ water)}$ before dilution. A dilution rate of 10 is used so values for $PEC_{effluent}$ have also been calculated and are in Table 8.2.

Table 8.2 - PECs calculated for the aquatic environment resulting from the use of limonenes in cleaning products (assuming release on 365 days of the year)

Isomer	Annual release to sewer (tonnes)	Emission to sewer (kg/day)	$PEC_{effluent}$ (µg/L)	PEC_{water} (µg/L)	
				High Dilution	Low Dilution
d-limonene	309	847	5.1	0.51	2.5
dipentene	23.7	65	0.39	0.04	0.19

Comparison with measured values

No Australian monitoring information was available from users or literature on this chemical in sewage discharge or groundwater to enable verification of the predicted environmental concentrations determined above. However, limonene has been detected overseas in groundwater and surface waters, ice, sediments, and soil. Mean limonene concentrations in two polluted Spanish rivers were 590 and 1600 ng/L. Samples of water collected from the Gulf of Mexico contained limonene at a concentration of 2 to 40 ng/L. Limonene has also been detected at Terra Nova Bay, Antarctica; water and pack ice samples contained limonene at concentrations up to 20 and 15 ng/L, respectively. Limonene concentrations up to 920 µg/g in soil and from 1 to 130 µg/L in groundwater were measured in a polluted area at a former site for the production of charcoal and pine tar products in Florida. It has also been detected at up to 20 µg/L in influent to a sewage treatment plant (STP) in Sweden but was not detected in the effluent from the STP - detection limit not stated (IPCS 1998). Limited data available suggest that the estimated PEC for Australia is rather high. However, it is likely to be much lower as limonene undergoes rapid biodegradation and evaporation.

8.1.4 Environmental concentrations in the atmosphere

When released to soil or water, limonene is expected to evaporate to the air to a significant extent, owing to its high volatility. This is underlined by Level 1 McKay modelling that indicates that 97.8% will partition to air at equilibrium. Thus, the atmosphere is the predominant sink of limonene. In the atmosphere it is expected that limonene will rapidly undergo gas phase reactions with photochemically produced hydroxyl radicals, ozone, and nitrate radicals.

Measured concentrations (between 1979 and 1992) of limonene in the air of rural forest areas in Europe, Canada, the USA, Nepal, the Republic of Georgia, and Japan ranged from 1.6×10^{-4} to 2.2 ppb (0.9 ng/m^3 to 12.2 ng/m^3) (IPCS 1998). Based upon these data, typical concentrations of limonene in air from rural areas range from 0.1 to 0.2 ppb (0.6 to 1.1 µg/m^3).

On the basis of measured concentrations (between 1973 and 1990) of limonene in the air from urban or suburban areas in Europe, the USA, and Russia that ranged from not detectable to 5.7 ppb (31.7 µg/m^3), typical concentrations of limonene in urban/suburban air are likely to range from 0.1 to 2 ppb (0.6 to 11.1 µg/m^3). Concentrations of limonene in air emissions from kraft pulp industries, stone groundwood production, and various waste and landfill sites have ranged from approximately 0.3 to 41 000 ppb (1.7 µg/m^3 to 240 µg/m^3) (IPCS 1998).

8.2 Occupational exposure

8.2.1 Routes of exposure

Occupational exposure to limonene in Australia may result from direct use of limonene either in end-use processes or during formulation of limonene products. Exposure to limonene may also occur during use of products containing limonene. Other potential sources of exposure are during transport and storage of the chemical and during disposal of contaminated containers.

In the assessment of occupational exposure to chemicals, it is generally necessary to evaluate intake from all potential routes of exposure i.e. ingestion, inhalation and dermal exposure. For limonene, ingestion is unlikely during occupational use except at workplaces with poor workplace practice and hygiene. An evaluation of information on use profiles indicates that Australian workers are potentially exposed through inhalation and dermal contact.

8.2.2 Methodology for assessing exposure

In order to assess potential exposure from use of limonenes in Australia, the available information on occupational use profiles obtained from suppliers and users of limonene and the information obtained from the NICNAS industry phone survey and site visits were evaluated.

No measured exposure data were made available for assessment by industry. There is no Australian occupational exposure standard for limonene.

Due to limited monitoring data for limonene, the EASE (Estimation and Assessment of Substance Exposure) model (version 2.0 for Windows) developed by the United Kingdom Health and Safety Executive (UK HSE) was used to estimate exposure during manufacture and formulation.

EASE is a knowledge-based electronic data system designed to facilitate the assessment of workplace exposure. It is used where measured exposure data is limited or not available. EASE predicts exposure as ranges in the form of conventional 8-hour time weighted average (TWA).

Exposure is determined by the EASE model at the high-end or maximum concentrations (i.e. worst case estimates) in feasible but not unrealistic situations (i.e. reasonable worst case situation). The estimates are not intended to be representative of extreme or unusual use scenarios that are unlikely to occur in the workplace. It is acknowledged that the EASE model takes a conservative approach and is likely to overestimate exposure. The majority of occupational exposures could be below these estimates.

EASE model assumes that the operator spends full shift (8 h) working at sites and is exposed to pure limonene alone. However, the majority of work processes involving potential exposure to limonene do not fit this assumption. Therefore the estimates were adjusted based on each use scenario and process description.

Three temperatures (10 °C, 20 °C and 40 °C) were modelled to cover the atmospheric changes in different seasons of a year. The results were the same at the three temperatures for dermal and inhalational exposure estimation. Also, information obtained from industry indicates that almost all work with limonene is carried out at room temperature. Therefore only results estimated at 20 °C are presented in the report. The input to the EASE model and the estimation before adjustment for the various scenarios are presented in Appendix 2.

Occupational exposure to limonene is discussed for each major activity with likely workplace exposure, namely:

- manufacture;
- importation;
- repacking;
- formulation; and
- use of limonene and products containing limonene.

8.2.3 Manufacture of limonene

d-Limonene is extracted in Australia by direct vacuum distillation from orange oil. It was confirmed that *d*-limonene would not be synthesised, for commercial reasons, because extraction from natural sources is easier and cheaper. Manufacture or extraction of *l*-limonene or dipentene in Australia was not reported.

At one plant, 200 L closed top steel drums of orange oil are transferred to the production area by forklift. Workers manually open the cap and insert a hose attached to the distillation system. The vacuum system sucks the orange oil into the distillation tank. The distillation process is a closed system and occurs under vacuum so that the limonene distils at approximately 40^0C to 90^0C rather than at the boiling point of 175^0C at normal atmospheric pressure. At the reduced pressure, water distils over first at 20^0C. Following distillation, clear *d*-limonene is produced and pumped into a clean closed top 200 L steel drum through another hose attached to the system. Limonene manufacturing is a continuous process and no sampling is conducted during distillation. Samples are taken from the drum either manually by using a stick with a small cup attached to the end of it or through a tap at the bottom of the steel drum for quality control purpose. Workers manually cap the drum and it is ready for sale.

The distillation residues (mainly waxes) are sold for waterproofing and to produce folded oils. Folded oils are concentrated materials with a strong odour left over after terpenes are removed and is cited in literature as being used in perfumery. Other wastes are collected by a waste management company.

The empty orange oil drums are cleaned either by high-pressure water cleaning or manual operation. They are re-used for end products. Cleaning of the distillation system does not occur very often and only undertaken between changes of products. It is usually cleaned by running terpene through the system for a couple of hours. Contaminated residues obtained after cleaning the system are collected by a waste company and the uncontaminated material is stored in an enclosed area and re-used for industry grade products. It was reported that minimal (trace) amounts of *d*-limonene is released to air during manufacture because of a trap system in the distillation system that ensures full recovery of *d*-limonene.

Limonene manufacture takes place continuously at some sites, but only once every 6 to 12 months at one site depending on market demand. In total 8 to 10 workers are involved in manufacturing activities at the three manufacturing sites identified. Extraction procedures are similar at the three sites. Gloves are normally used. Masks/respirators are used when cleaning drums or whenever there is a strong odour.

An air conditioning system is fitted on the roof at one of the 3 sites and general ventilation was reported at the other 2 sites. It is possible that *d*-limonene production occurs at other sites in Australia but not reported to NICNAS.

Potential exposure to limonene during manufacture is limited due to the enclosed system. Exposure may occur during sampling after manufacture of *d*-limonene.

Measured exposure data

Static air monitoring of *d*-limonene was conducted at one of the manufacturing sites as part of a quality control program a few years ago. A sensor placed at one of the production areas detected 'very low' levels of *d*-limonene. The monitoring report was not provided by the company. No personal air monitoring information during manufacture of *d*-limonene is available in Australia or overseas.

Estimated data

The EASE scenario that best describes the manufacturing process is closed system without direct handling and system breaching as it refers to processes in which substances remain in an enclosed system (UK HSE, 2000). The predicted dermal exposure to limonene during manufacture is very low and inhalational exposure is estimated as 0-0.1 ppm (0-0.56 mg/m^3).

8.2.4 Importation

Most of the *d*-limonene and *dl*-limonene are imported in 200 L UN approved epon-lined steel drums, 173 kg to 175 kg nett, new mild steel drums or in 1 L containers for laboratory uses. The materials are transported mainly by road from the arrival port to the storage warehouse or to customers directly, using carriers licensed to carry Class 3 flammable goods. In the case of storage, all notified importers store limonene in an approved Class 3 flammable store. According to MSDS submitted by industry, limonenes are stored in a cool, well ventilated area, out of direct sunlight and away from sources of heat or ignition and from oxidising agents, acidic clays and mineral acids. The temperature is kept below 27°C and containers are closed at all times. Regular checks for leaks are conducted.

It was reported that the disposal of unused imported limonene at storage sites is handled by a contractor licensed to dispose Class 3 flammable liquids. No information is available on the total number of workers handling limonene during importation and transportation.

Occupational exposure during importation, transportation and storage is unlikely except in cases of accidental spills or leaks of the chemicals.

Importation of products containing limonene

Information obtained from industry indicated that products containing *d*-, *l*- and *dl*-limonene are imported in a variety of package sizes depending on different package types. The package sizes of intermediates such as perfume blends vary between 25 to 205 L steel drums. For end products imported and sold to the general public directly, the package sizes range from 50 g to 3 kg and from 75 mL to 4 L. The products are transported either by a normal transport company or Dangerous Goods approved transport company depending on the label requirements. They are transported mainly by road from the arrival port to the warehouse and/or to customers. The products containing limonenes are stored in a general warehouse or in a flammable storage area for products classified as dangerous goods. The products are reported to be stored away from sources of ignition, in a cool and dry area or in a well ventilated, undercover building i.e. a bunded drumstore.

Information obtained from industry indicated that products containing limonenes are mostly disposed of via licensed trade waste companies. Some companies dispose of limonene products by evaporation or incineration following EPA and local regulations. No disposal occurs in some companies as rejected material is returned to the suppliers.

Exposure of workers to the products is limited except in cases of accidental spills or leaks of the products. Some companies reported that all workers wear protective clothing and use protective gloves and eyewear as part of normal chemical handling practice.

8.2.5 Repacking

Repacking of both manufactured and imported limonene and products containing limonene occurs in Australia.

According to the information submitted by industry, most repacking of limonene is from 200 L steel drums to smaller containers such as 5 L and 20 L drums. Limonene is decanted into smaller containers either through a pump (enclosed process) or manually i.e. operator puts a tap on the opening of the 200 L drum and manually tips limonene out into another drum. Usually one operator is involved in this activity at each site. Repacking is usually not a continuous operation and occurs about 1 to 4 times a month at most of the worksites depending on customer orders. It was reported that workers wear gloves and safety glasses during this process.

Limonene is also repacked from large storage tanks with capacities of up to 14.5 tonnes. The material is pumped into the tanks and transferred through a tap at the bottom of the tanks with a quantity control device, into 200 L drums or smaller containers. Usually 1 operator is involved in this process, which occurs 3 to 4 times a month depending on customer orders. Sufficient containment to cover spillage of all of this through bunding and a sump is in place. The storage tanks are also used for other non-limonene materials and are often not cleaned between different products, as the tanks are conical with drainage points and are self-draining. However, they are cleaned if "organic" material is put in the tank. During cleaning workers enter the tanks and wipe it out or flush with alkaline materials (< 5%). Rubber gloves and goggles are used in this process, and the caustic is considered to be the main hazard. Respirators are not used during cleaning.

Repacking of products containing limonene occurs either directly after the products are manufactured/imported and then used for further formulation or before end use. The package sizes before repacking vary largely and repacking methods also differ from company to company. For example, a product 'Hand Cleaner' containing 8% *d*-limonene is repacked from commercial pack sizes of 5 L into smaller packs (500 ml pump pack) through a tap at the bottom of the 5 L container and sold for consumer use.

No information was available on the total number of workers handling limonene during repacking. The duration of the repacking activities varies depending on market demands.

In summary, exposure of workers to limonene during repacking of limonene or products containing limonene is limited as this is a periodical process at most sites, especially when this is undertaken in an enclosed system. Operators are likely to be exposed by skin and eye contact during manual pouring and equipment cleaning. There is also a potential for inhalation exposure, especially when working in a confined space during cleaning of a storage tank for repacking.

No overseas and Australian measured exposure data for repacking of limonene or products containing limonene are available.

8.2.6 Formulation

In Australia, limonene is used mainly in formulating a range of industrial and consumer products including flavour and fragrance additives.

Information on formulation was obtained from industry submissions, site visits and the NICNAS industry phone survey. To investigate downstream handling and use of limonene, approximately 80 companies were selected for the phone survey from customer lists submitted by manufacturers and importers of limonene. As far as possible a range of industry sectors and end uses were included in the selection. In total 41 formulators were contacted by phone and participated in the survey. The information collected during the survey included product details, description of formulation process, personal protective equipment, current controls and potential release to environment. The survey forms used for the phone survey are in Appendix 1.

Formulation process

Although limonene is used to formulate a large number of products in various industries the general formulation procedure is similar. A number of examples are selected from industry submissions and the NICNAS industry survey of formulators and presented in Table 8.3. In general, formulation is a batch process, in which measured amounts of limonene and other components are added to mixing vessels and blended to form products, which are transferred to containers and then dispatched to customers. Batch sizes and intervals vary from site to site.

The formulation process varies depending on size of production. In general, for small batches, limonene is decanted into another vessel through a tap either on top or at the bottom of the container for weighing and then manually poured into a large tub and stirred. Samples are usually taken manually at the end of mixing with a cup. Decanting is done manually with a small jar and funnel. Equipment is cleaned manually between different products with either water or cleaning solvents.

For larger-scale production a 200 L drum of limonene is either directly poured into a mixing tank by a drum lifter or is transferred via a transfer pump. Other ingredients are then added followed by mechanical stirring. Antioxidants are used in some products. For some formulations, limonene is premixed with other ingredients before adding into the main mixing vessel. The premixes are heated at some worksites in order to melt or dissolve individual ingredients. Only one formulator reported applying heat to 60 °C during the main mixing operation. The mixing operation is usually conducted under closed conditions and the final product pumped into large drums for shipping to customers. The mixing process usually takes 0.5 h to 2 h depending on quantity of the batch and the ingredients. Some companies reported that the batch operation takes half to one day. Operators are not in attendance for most of the mixing time and rely on periodic inspection. Sampling is conducted at the end of mixing and samples are usually taken through a tap at the bottom of the mixing vessel. Equipment is cleaned manually between different products by hosing pressured water or using cleaning solvents. Decanting is usually an automated process, but one formulator reported that manual decanting is done for alcohol-based products.

No information was available on the total number of workers handling limonene during formulation, as only a proportion of formulators would have been contacted by the NICNAS industry survey. In the majority of workplaces surveyed, one operator is involved in each of the process (weighing, loading and mixing) during each batch. A number of workers are involved in the decanting process depending on batch size.

The final formulated products are packed into different types of packaging such as steel cans, pails, drums or tubes and stored at warehouses according to the product Dangerous Goods classification. Most products are not classified as Dangerous Goods and are stored in general warehouses. Some companies reported that products are stored in bunded areas so that spills can be contained within the site and not released to the general environment. The formulated products are transported mainly by road using licensed and correctly placarded vehicles in accordance with the Australian Code for Transportation of Dangerous Goods by Road and Rail (FORS, 1998).

Usually no waste is generated as exact amounts of limonene are used to formulate products. If any wastes are formed they are disposed in accordance with disposal procedures. In some companies, waste product is stored in drums and sent to a third party when sufficient quantities are collected.

d-Limonene drums are sold to drum reconditioners for reprocessing. Products or packaging containing *d*-limonene for disposal is normally sent to the relevant waste authority disposal depot.

The majority of formulators reported that workers wear safety glasses and gloves when handling limonene and products containing limonene. At some workplaces workers are required to wear overalls, safety footwear and apron. Masks and full-face visors are worn when necessary.

Exhaust ventilation above the mixing tank is used at the majority of the workplaces. Some sites rely on natural ventilation.

In summary, during formulation of products containing limonene, operators are likely to be exposed by skin contact during manual charging of mixing vessels, mixing and inspection, filling of product containers and cleaning equipment. There is also a potential for inhalation exposure, especially during heated blending process and high speed mechanical stirring. The potential inhalation exposure of workers during formulation is likely to be higher at worksites with an open mixing process and no exhaust ventilation. However, since formulation is a batch process, exposure will

...ncy	PPE	Controls
...every ...ks	Overalls, gloves, boots and safety glasses	LEV above each mixing tank
...batch ...th	Gloves, safety glasses, overalls, boot, hairnet, mask for people with facial hair	Floor type exhaust ventilation
	Gloves, masks when necessary	Fume extraction and carbon filter system
	Apron, gloves, overall and glasses	LEV above mixing tank
	Safety glasses, boots, gloves and overalls	NR
	Gloves, safety glasses	Natural ventilation
...onth	NR	NR
	Safety glasses, boots, gloves and overalls	LEV (3 years old)

Table 8.3 - Examples of formulation of limonene products, with control measures

Measured exposure data

No overseas and Australian measured exposure data for formulation of products using limonene are available.

Estimated data

Dermal exposure

Surfaces of equipment may remain contaminated with limonene and/or limonene products as limonene has low volatility. This may result in dermal contact during handling of equipment. The EASE model that best describes this scenario is non-dispersive use with intermittent contact. According to the EASE model, intermittent contact is assumed to be 2 to 10 events per day involving exposure as part of a process. This results in an exposure of 0.1 to 1 mg/cm^2/d.

Exposure by inhalation

It was reported that local exhaust ventilation (LEV) systems are used above mixing vessels at some of workplaces, but the majority of workplace rely on general ventilation. For the purposes of modelling occupational exposure to limonene, two scenarios were considered:

1. plants operating with LEV;

2. plants operating without LEV but with segregation.

According to the EASE model, segregation indicates separating the worker from the substance by distance, typically a few meters from the source of exposure, which suits the formulation process described above.

The EASE scenario that best describes the formulation process is non-dispersive use as it refers to processes in which substances are used in such a way that only certain groups of workers, with the knowledge of the processes, come into contact with these substances (UK HSE, 2000).

Inhalation exposure to limonene vapour by the EASE model is estimated as 0.5 to 1 ppm (2.8 to 5.6 mg/m^3) and 3 to 5 ppm (16.7 to 27.8 mg/m^3) for plants with LEV and with segregation, respectively.

Adjustment of the estimation

For dermal exposure, the operator comes into contact with 100% limonene only during charging and sampling. In addition, all companies responding to the NICNAS industry survey reported using gloves and other types of personal protective equipment (PPE). It is therefore reasonable to assume that the exposure is at the lower level of the above range, i.e. 0.1 mg/cm^2/d.

For exposure by inhalation, information obtained from the NICNAS industry survey indicates that formulation takes about 0.5 to 2 h in the majority of workplaces and the operator is likely to be exposed to pure limonene only during weighing and charging of limonene into the mixing vessel. LEV is in place at most formulation sites surveyed. It is therefore reasonable to assume that the operator is only exposed to limonene for one quarter of the shift. The EASE prediction can therefore be adjusted for the period of no exposure and this results in an 8-hour TWA of 0.125 to 0.25 ppm (0.7 to 1.4 mg/m^3) for plants with LEV and 0.75 to 1.25 ppm (4.2 to 7.0 mg/m^3) for plants with segregation.

8.2.7 Use of limonene and products containing limonene

Information on end use of limonene and products containing limonene were obtained mainly through the NICNAS industry survey. Twenty-five out of 40 formulators completing the formulator survey were selected based on the type of products, to identify as many end uses as possible. These formulators were asked to provide 2 to 5 customer contact details to NICNAS for description of end use. In total, 22 end users completed the survey. Information sought by NICNAS end user survey included detailed use process, PPE and control measures. The survey forms used for the phone survey are presented in Appendix 1.

Tables 8.4 and 8.5 detail information on the use of limonene and products containing limonene, respectively. In general, the use of limonene and products containing limonene involves manual handling under open conditions. The applications of limonene and products containing limonene vary largely and include:

- spraying;
- brushing;
- pour in and pour out;
- bath/dipping;
- wipe on and wipe off;
- mopping/hosing; and
- personal application in the case of heavy-duty hand cleaners.

The duration of use varies from a couple of minutes per day to continuous use depending on the feature of work. Information on the total number of workers in Australia using limonene or products containing limonene is not available.

All companies reported use of gloves and some companies also use other PPE such as safety glasses, overalls, safety boots, masks or respirators. The majority of workplaces use limonene or limonene products under general ventilation. The use of LEV was reported at some workplaces.

In summary, information on the range of end uses and potential exposure is limited due to the relatively small number of end use respondents to the NICNAS survey. However, based on the widespread end uses, modes of use and lack of control measures at some worksites, end use of limonene or limonene products gives the greatest potential for worker exposure.

Table 8.4 - Description of uses of limonene

Uses and Use Process	No. of Workers	Duration	Frequency	Engineering Controls	PPE
Glass mould cleaning Used in a purge spray to clean out graphic coatings in glass mould. Cleaning residues are collected in 20 L drums that go to a licensed contractor for disposal.	1	2 min 3 h/d	Daily	Exhaust ventilation	Gloves, goggles and protective clothing
Removal of wax from laboratory slides prior to mounting Used in tissue processing machines as a cleaning agent between alcohol and paraffin wax, removing wax from slides and as a cleaning agent for cover slips and slides for microscopy work. It is applied automatically. Equipment cleaning (dishes, glassware etc.) is by vaporisation followed by normal glass washing procedures. Machinery is designed to withstand the chemical so no need to clean.	Varies (2-24)	24 h/d	Daily	Fume extraction, biological and bench fume hoods	Latex gloves. Trialing nitrile gloves now.
Cleaning of containers such as tanks with viscous residues The product is manually poured into tanks, which are then covered and stored. The time that limonene is left in tanks varies depending on the viscous residues. The limonene is then manually poured into a drain and run through a water treatment plant where limonene is treated in the same way as oil/grease. Ferric chloride is used as an emulsion breaker and the oily layer is skimmed from surface of the treatment vessel and taken off site. The concentration of limonene in the waste stream is about 0.8%.	2-3	10-20 h/d	210-220 d/year	Exhaust and general ventilation	Gloves, safety glasses, apron, overall, gum boots, face mask (sometimes)
Polystyrene cleaning (stripping) Compressed limonene in pump spray pack is sprayed onto styrene foam to reduce it to a paste to aid with disposal. Equipment used is cleaned with high-pressure water and the cleaning residues are stored and taken to a local hazardous waste plant.	2	1 h/d	twice/week	In open air on breezy day only	Coveralls, gloves, respiratory and eye protection
Rubber stripping Limonene is poured manually into a mixer with rubber materials, left for a period of time and then manually poured out (pour-in and pour-out process).	Unknown	Unknown	Once only	Unknown	Unknown
Cleaning of aircraft part Limonene in 44-gallon drums are decanted manually into 5 L or 100 mL containers and applied on aircraft parts by manual spraying or brushing. Some parts are dipped in a tank with limonene for up to 1 day. Limonene is wiped off the parts with rags/cloths. The rags/cloth are left on the floor for fumes to vaporise and then sent to waste.	170	5 mins to 1 day	Varies (twice/year to weekly) depends on the job	Big open area	Gloves, glasses

Table 8.4 - Description of uses of limonene (Cont.)

Uses and Use Process	No. of Workers	Duration	Frequency	Engineering Controls	PPE
Microbiological testing Limonene is used as part of confirmatory tests. It is not diluted and is used to decolourise slides with the aid of disposable pipettes and forceps. The disposable pipettes are autoclaved, so no residue is left as it evaporates off. Beakers used to collect used material are rinsed with cold tap water and placed in dishwasher for thorough cleaning.	2	30 min	Once in the last 5 months for training purpose	Fume cupboard for decolourising slides	Plastic and Latex gloves, cotton uniform
Wax removing Used in beauty salons to remove anything contaminated with wax such as the outside of wax pots, carpet and floor etc. A cotton wool or cloth dipped with the product is used to manually wipe the contaminated surface and is then thrown into the general bin.	1	1-2 min	Once/d to weekly depending on business	General ventilation	Gloves
Gem testing About 1 L *d*-limonene is manually poured from a 25 L drum into a glass container in an open-air area. Gemstones are immersed in the solution for about 5 min and then observed indoor using a microscope for a couple of minutes. The gemstones are manually taken out of the solution and wiped with tissue. The solution is poured back to the 25 L drum and sealed and stored for reuse. The glass container is wiped with tissue and then rinsed with warm water. The contaminated tissue goes to general bin.	1	10 min	Once/week	Open air and general ventilation	Surgical gloves
As an adhesive agent for tyre re-treatment Dipentene is poured manually into a small bath fitted with a sponge-covered roller. During processing the roller picks up dipentene in the bath and coats the cooled rubber sheet. The rubber sheet is then pressed and joined onto a plastic sheet. The dipentene reacts with the rubber in 24 h making the surface sticky. The processed rubber is rolled up mechanically and ready for tyre re-treatment.	3-4	5 min (from the time the rubber goes through the dipentene bath to runs out of the extruder)	4-5 h/d, once/month	Open area	Goggles, gloves and overalls that are washed on site
Degreasing (lubricant removal) in metal forming industry Limonene in 200 L drums is decanted into smaller containers before use. A worker takes a wad of waste cloth, soaks it in limonene and rubs on metal surface. The used wad of cloth is sent to compactors and goes to landfill waste.	1	30 min	1-2 d/month	Roof fan (1 year old)	Rubber gloves, masks, laundered overalls

Table 8.5 - Description of uses of some limonene products

% LIM in Product	*Uses* and Use Process	No. of Workers	Duration	Frequency	Engineering Controls	PPE
65-95	***As a degreasing solvent for munitions, bullets etc.*** The product is dabbed on with a rag or paintbrush and at times munitions are submerged in the product overnight. About 20-30 L/month cleaning residues are burnt on site and the residues are sent to landfill. Spills are wiped with rags, which are burnt.	< 10	Seconds to overnight	Depends on orders	Exhaust ventilation (< 7 years old)	Gloves, safety glasses, full length clothing
80	***Bitumen removal in laboratory*** The product is poured on a cleaning rag that is used to wipe down benches and sinks that may have bitumen. Cleaning rags and paper towel are sent to normal waste disposal.	1	2-3 min	Once per day	Air conditioning system, fume cupboards and fume extraction over sinks	Safety glasses, nitrile gloves, laboratory coat, safety shoes, wrist to ankle clothing
30	***Used to remove texta graffiti*** Small quantities of the product (1-2 egg cups) are put in a bucket and brushed onto the surface and wiped off with a clean damp cloth. Cleaning cloth is washed in an ordinary washing machine and re-used.	2	Varies	Daily	Mostly outdoors	Gloves, safety glasses, organic respirators for working in confined space
25	***1) Tar/bitumen removal from trucks*** The product is diluted with water and mixed with other detergents. The concentration of limonene in the final solution is 10-15%. It is broomed, mopped or hosed onto trucks. The washings from trucks go to special sumps that contain all the chemical by-products.	NA	NA	1 h/week	NA	NA
	2) Machinery degreasing The product is decanted into a 5 L pressure sprayer. It is sprayed onto machinery and left over night and cleaned off with a pressure cleaner. Cleaning residues and any drips are washed into a pit/tank that goes to an oil separator.	4-5	4-5 min	Used once only	Open area on a concrete floor	Gloves
20	***Wire rope lubricant*** This product is used in automatic or manual industrial lubricators, whether pump force feed, wick feed, drip feed or brushed. Ideally the lubricant should be applied close to the point where the strands of the rope tend to open when passing over a sheave or drum (from a technical data sheet provided).	NA	NA	NA	NA	NA

Table 8.5 - Description of uses of some limonene products (Cont.)

% LIM in Product	*Uses* and Use Process	No. of Workers	Duration	Frequency	Engineering Controls	PPE
17-18	***Hand cleaner*** Apply small quantity of the product to hands and work thoroughly into skin. Rinse off with water or wipe clean with a cloth or paper towel.	Unknown	1-2 min	About 5 times/d	N/A	N/A
15-35	***Cleaning printing press blankets and rollers*** The product is decanted into a bucket and manually applied onto printing press blankets and rollers with a rag. Cleaning rags are washed with tap water which is emptied into the drain and rags re-used.	2	7-8 min	2-5 times/d and daily	Ventilation fan	Gloves
5	The product is used in an automatic washing system and empty containers go to waste dumper (provided by a supplier).	NA	NA	NA	NA	NA
10-20	***Wool/fabric wash*** The product is weighed and added manually to a tank next to a machine holding 5000 L water. It is then pumped into the machine to make a final solution containing 0.01-0.02% limonene. Dirty wool/fabrics are loaded and mechanically stirred in an enclosed system for 1-2 h at 50-80 °C. The solution is then released through a pipe to a holding tank where it is monitored by EPA and disposed according to regulations. The wool/fabric is washed, rinsed with water and loaded manually into a dryer.	1-18	Few minutes	Varies but usually 1-2 times/week	Exhaust ventilation at weighing area and general ventilation at machine area	Gloves, safety glasses, protective clothing
2	***Automotive base coats in crash repair*** End users add thinner to the paint before use (ratio unknown). It is applied by spraying.	Unknown	1-30 min depends on size of job	2-10 cars/d depends on size of workshop	Unknown	Unknown
0.5	***Used in making scented plastic bags*** The scented pellets are automatically added to a resin mix and mixed in a hopper, then the mixture is dropped through a screw into a barrel where it is heated to about 220-230 °C. It passes through a film extruder and is used to make plastic bags. Equipment is purged with another master batch with a binding agent. Residues are discarded to hard scrap.	3-4	24 h/d	5 d/week	Unknown	Protective clothing and hat. Gloves and masks are optional

NA, not available.

Measured exposure data

No Australian measured exposure data during use of limonene or products containing limonene are available.

There are a number of published overseas studies reporting levels of limonene from personal and static sampling. The measurements were made during industrial use of limonene and limonene products in graffiti removal, metal stripping, aircraft parts degreasing, electronic assemblies cleaning, printing work and the information is summarised in Table 8.6. Limonene was also detected during monitoring of limonene containing substances in some workplaces and the information is shown in Table 8.7.

Personal monitoring of workers involved in graffiti removal, metal stripping and aircraft parts degreasing over 8 h measured levels of limonene ranging from 0.02 to 3158 mg/m^3 (0.003 to 559 ppm) (Anundi et al., 2000; Clark et al., 1997; Kiefer et al., 1994). The lowest level was measured at graffiti removal when limonene was used in open spaces in aerosol form and the highest level was detected during metal stripping when the products containing limonene were used in enclosed areas with limited ventilation. In the monitoring study by Kiefer et al (1994), a level of up to 634 mg/m^3 (112 ppm) was measured during aircraft parts degreasing when the parts were soaked in products containing limonene and were manually brushed and rinsed. A level of 22.7 mg/m^3 (4.0 ppm) was measured on a printing operator using a products containing 50% limonene, but there was no information on number of samples, duration of the sampling, engineering controls and PPE used (Josefsson, 1993). The levels of limonene around workers' breathing zone ranged from 100 to 200 mg/m^3 (17.7 to 35.4 ppm) over 8 h period in a few workplaces where monoterpene including limonene was produced (Svedberg & Galle, 2000; Rosenberg et al., 1999; 2002).

Static monitoring measured levels of limonene ranging from 0.56 mg/m^3 (0.09 ppm) measured near a solvent tank to 400 mg/m^3 (70.8 ppm) when metal is cleaned by high-pressure washing.

8.3 Public exposure

8.3.1 Consumer exposure

Limonenes are present in foods, occurring naturally in citrus fruits, vegetables and herbs (celery, celeriac, fennel, dill), and when used as a food flavour and fragrance additive (at $\leq$ 1%). The principal source of public exposure is by ingestion of foods containing limonene. The intake of *d*-limonene in the USA from food was estimated to be 0.27 mg/kg/day (IPCS, 1998).

The potential for public exposure to limonene during manufacture, importation, transport, storage, and industrial use is expected to be low.

Limonenes are also present in many consumer products for their solvent (cleaning) and/or fragrant properties (usually citrus). The diversity of such consumer products includes medical devices, personal hygiene products, baby products, first-aid products, medicinal cosmetics, sunscreen products, bath products, anti-perspirants, deodorants, cosmetics, hair products, lip products, soaps, detergents, body lotions, perfumes, colognes, washing powder and liquids, animals products, and products such as plastics and air-fresheners. Solvent-type cleaners (for removing gum, grease, tar, etc.) contain between 20 and 60% limonene. Fragrance blends can contain up to 90% limonene, with dilution in finished products to 0.002% to 0.2% in (ascending order of concentration) dishwashing liquids, shower gels, body lotion, liquid detergent, shaving foam, deodorants, and in perfume sprays. Limonenes are contained in many “essential oils” at between 0.1 and 96% and are used in aromatherapy, as environmental fragrances (in oil burners), for therapeutic purposes involving inhalation and dermal contact, and have food and cleaning uses. Limonene is present in shoe finish products at 15 to 16%, and is used as insect attractants in household pesticides and in timber finishes. Dipentenes are generally used as “pine “ fragrance in consumer products and are used in shaving foam, foam carpet cleaner, leather and vinyl cleaner, furniture polishes, and laundry powders, at 0.001 to 0.08%.

Consumer exposure will be widespread and will predominantly occur by ingestion and dermal contact with foods and inhalation of indoor air containing limonene. There will also be inhalation and dermal exposure, with the potential for oral ingestion and ocular contact, from the use of consumer products containing limonene.

Dermal exposure from consumer products containing limonene can be estimated using typical use levels adopted from the EC technical guidance document (European Commission, 1996). From the consumer products outlined in 7.5.3, daily use of non-rinse products such as perfume spray, deodorant spray or roll-on, or body lotion would involve approximate exposures of 0.225, 0.063, 0.010, or 0.02 mg/kg/day limonene, respectively. For daily use of rinse-off products, such as shower gel and shaving foam, exposures would be 0.001 and 0.002 mg/kg/day limonene, respectively. Exposure via a hand cleaner containing 15% limonene could be up to 1.25 mg/kg per use assuming 10% is left on the skin after wiping or rinsing. Exposure to other products, such as furniture/floor polish (70% limonene), shoe finish product (16% limonene), household cleaner (1.9% limonene), and bug/tar remover (22% limonene) would maximise at around 0.08 mg/kg/day, when used once per week. Combined exposure from a person using limonene containing shower gel, shaving foam, body lotion, deodorant spray, hand cleaner (non-rinsed), and household cleaner every day would be approximately 0.24 mg/kg/day.

Table 8.6 - Summary of overseas monitoring data on limonene

Use	% LIM	Type of sampling	No. of samples	Duration	Location/worker activity	Ventilation	PPE used	Sampling method	Sample analysis	Result (mg/m^3)	Reference
Graffiti removal	100	Personal	38	15 min – 8 h	Spray on and wipe off (small area) Brush on and spray off (large area)	Open air, poorly ventilated areas such as underground stations or train carriages and enclosed spaces such as elevators	Yes	Charcoal tubes	GC	0.02–0.52 (8h TWA) 0.21 (15 min STEL)	(Anundi et al., 2000)
Metal stripping	Unknown (3 products)	Personal	26	10-16 min	Metal refinishing (removal of old lacquers and spraying on of new coatings)	Enclosed areas with poor ventilation such as elevators	Yes	Charcoal tubes	GC	100-3158 (8h TWA) (5 samples > 834)	(Clark et al., 1997)
Aircraft parts degreasing	20 and 86 (2 products)	Personal	8	70 min to 7.5 h	Soaking, brushing and rinse/drying	Not stated	Yes	Charcoal tubes	GC-FID	0.78-634	(Kiefer et al., 1994)
		Static	7	23 min to 7.5 h	Near solvent tanks	Not stated	N/A	Charcoal tubes	GC-FID	0.56-64.4	
Metal degreasing before industrial painting	6 (factory 1)	Static	Not stated	Not stated	Metal dipping into a built-in bath – measurement around the bath	Not stated	Not stated	Not stated	Not stated	0.9-6	(Josefsson, 1993)
	2-3% in bath 7% in washing product (factory 2)	Static	Not stated	24 h	1) Manual degreasing in an open bath 2) high pressure washing	Not stated	Not stated	Not stated	Not stated	50 (average) 1) 80-100 2) 400	
Electronic assemblies cleaning	46% in bath	Static	Not stated	Not stated	1) Around bath 2) During manual handling	Not stated	Not stated	Not stated	Not stated	1) 10 2) 200	(Josefsson, 1993)
Printing work	50	Personal	Not stated	Not stated	Not stated	Not stated	Not stated	Not stated	Not stated	22.7	(Josefsson, 1993)
		Static	Not stated	Not stated	Not stated	Not stated	Not stated	Not stated	Not stated	69.7	

Table 8.7 - Summary of overseas monitoring data on limonene containing substances

Workplace (Substance monitored)	% limonene in substance	Type of sampling	No. of samples	Duration	Location/worker activity	Ventilation	PPE used	Sampling method	Sample analysis	Result (mg/m^3)	Reference
3 sawmills processing pine and spruce (monoterpene)	Pine processing (2%)	Personal	89	3 consecutive days	Sawmill workers (Sawing and edging)	LEV and general ventilation	Not stated	Diffusive sampling using charcoal	GC-FID	61-138 (GM) (8 h TWA)	(Rosenberg et al., 1999, 2002)
					Other workers in sorting, stacking, grading and packaging					0.47-33 (8 h TWA)	
		Static	46	8 h for 3 consecutive days	General area	LEV and general ventilation	N/A	Diffusive sampling using charcoal	GC-FID	161-193	
	Spruce processing (6%)	Personal	90	3 consecutive days	Sawmill workers (Sawing, edging)	LEV and general ventilation	Not stated	Same as above	GC-FID	2-13 (8 h TWA)	
					Other workers in sorting, stacking, grading and packaging					0.37-4.8 (8 h TWA)	
		Static	36	8 h for 3 consecutive days	General area	LEV and general ventilation	N/A	Same as above	GC-FID	16	
Sawmills (monoterpene)	2.5	Personal	7	1-3 h	No details	Dust extraction system	Not stated	Charcoal tubes	GC	166± 47	(Svedberg & Galle, 2000)
		Static	Not stated	85 h	2 shifts a day (18 h)	Dust extraction system	N/A	Long path FTIR	GC	166± 47 (peak value 260)	

Table 8.7 - Summary of overseas monitoring data on limonene containing substances (cont.)

Workplace (Substance monitored)	% limonene in substance	Type of sampling	No. of samples	Duration	Location/worker activity	Ventilation	PPE used	Sampling method	Sample analysis	Result (mg/m^3)	Reference
Compost swineries (terpene)	Unknown	Static	Not clear	Not clear	At a height of 1.5 m on the aisle in the middle part of the swinery	Not stated	N/A	Adsorption/ thermal desorption method	GC/MS	1	(Louhelainen et al., 2001)
Bakery shop (VOC)	N/A	Personal	1	5 h	Shrink-wrap machine operator	LEV	Not stated	Charcoal tubes	GC-FID	0.2	(NIOSH, 1989)
		Static	2	6 h	Directly over a heat sealer/cutter bar on each of 2 units	LEV	N/A	Charcoal tubes	GC-FID	0.2	

GM, geometric mean; LEV, local exhaust ventilation; VOC, volatile organic chemicals; N/A, not applicable.

8.3.2 Exposure via environment

Limonenes are monoterpenes and occur in plants, including commercially important trees and bushes, fruits, vegetables, and herbs, and in turpentine. *d*-Limonene is the main constituent of oil from citrus fruits (> 90% orange, 90% grapefruit, 70% lemon). *l*-Limonene is mainly found in pine needle oils and turpentine, but also in spearmint and peppermint.

Significant quantities of monoterpenes are released into the atmosphere, although no information on monoterpene levels in Australia is available. Air concentrations of limonene and other monoterpenes can vary considerably, depending on variations in the weather, seasons, and vegetation.

Limonene is present in indoor air. Measurements of total volatile organic chemicals (TVOCs) in Australian (Commonwealth Scientific Industrial Research Organisation, CSIRO) buildings varied from 32 to 143 $\mu g/m^3$ in which the major VOC was limonene (outdoor TVOC were 9 to 27 $\mu g/m^3$). Interestingly, there were no complaints in the building with the highest TVOC (143 $\mu g/m^3$) consisting mainly of limonene, alkanes, and pinene, whereas there were complaints in a building with a TVOC of 32 $\mu g/m^3$ consisting mainly of limonene and acetone. It seems therefore that the identity of indoor VOC's is at least as important as the TVOC levels.

Limonene has been detected in ground and surface waters, sediments and soil. Intake from drinking water is likely to be low due to the low solubility of limonene.

The main routes of public exposure from environmental sources is likely to be widespread, the main routes of exposure being inhalation and dermal contact.

9. Comparative Kinetics and Metabolism in Laboratory Animals and Humans

Sections 9,10 and 11 of this report have been scanned from the IPCS CICAD report on limonene (IPCS, 1998), a major source of information for the assessment. The literature search carried out for the IPCS covered 1996-1997 and the summary has been supplemented by new data where found. References in Sections 9, 10 and 11 that have not been sighted have been marked with an asterisk.

d-Limonene has a high partition coefficient between blood and air ($\lambda_{blood/air}$ = 42) and is easily taken up in the blood at the alveolus *(Falk et al., 1990). The net uptake of *d*-limonene in volunteers exposed to the chemical at concentrations of 450, 225, and 10 mg/m^3 (79.6, 39.8, and 1.77 ppm) for 2 h during light physical exercise averaged 65% *(Falk-Filipsson et al., 1993). Orally administered *d*-limonene is rapidly and almost completely taken up from the gastrointestinal tract in humans as well as in animals *(Igimi et al., 1974; Kodama et al., 1976). Infusion of labelled *d*-limonene into the common bile duct of volunteers revealed that the chemical was very poorly absorbed from the biliary system *(Igimi et al., 1991). In shaved mice, the dermal absorption of [^{3}H] *d/l*-limonene from bathing water was rapid, reaching the maximum level in 10 min *(von Schafer & Schafer, 1982). In one study (one hand exposed to 98% *d*-limonene for 2 h), the dermal uptake of *d*-limonene in humans was reported to be low compared with that by inhalation *(Falk et al., 1991); however, quantitative data were not provided.

In vitro permeability studies using hairless mice skin pre-treated with terpene in propylene glycol for 1 h indicate that limonene and other terpene compounds enhance dermal penetration of drugs (Godwin & Michniak, 1999). The enhancement effect varied with different drugs and different terpenes. Earlier studies suggest that hydrocarbon terpenes such as limonene are effective in enhancing the penetration of lipophilic drugs. The authors suggest that limonene distributes or attacks the skin surface quickly, as maximum enhancement is reached after only 1 h pre-treatment, and that the mechanism of action of terpenes involves disruption of the intercellular lipids of the stratum corneum.

Studies of the permeability of isolated human epidermis and dermis to *d*- and *l*-limonene and dipentene indicated that the dermis did not act as a barrier, whereas penetration was slower in the presence of epidermis. Release and penetration through the dermis and epidermis were as least 3-4 times faster for dipentene than for the *d*- or *l*- isomers. Large amounts found in epidermis suggested a high affinity of the compounds to the stratum corneum (Cal et al., 2001).

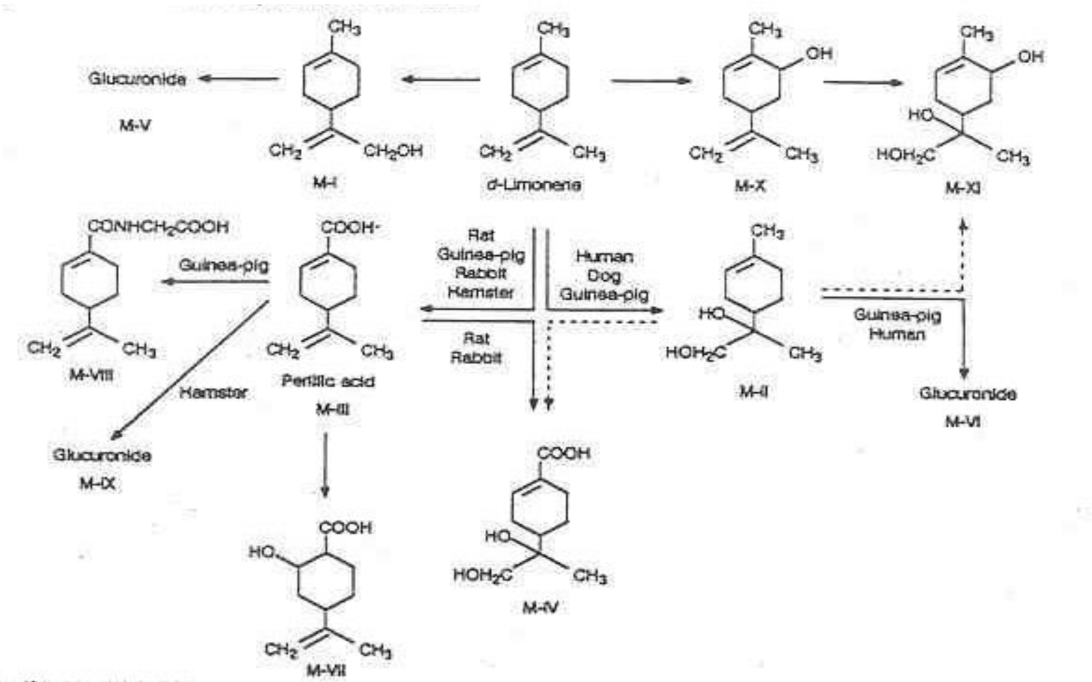
d-Limonene is rapidly distributed to different tissues in the body and is readily metabolised. Terpene hydrocarbons such as limonene (and terpene ketones) accumulated at a greater rate in the brain than alcohol, aldehyde and phenol terpene compounds in mice exposed to essential oil vapours in closed boxes (Inoue & Yamaguchi, 2000). Clearance from the blood was 1.1 L/kg bw per hour in males exposed for 2 h to *d*-limonene at 450 mg/m^3 (79.6 ppm) (*(Falk-Filipsson et al., 1993). A high oil/blood partition coefficient and a long half-life during the slow elimination phase suggest high affinity to adipose tissues *(Falk et al., 1990; *Falk-Filipsson et al., 1993). In rats, the tissue distribution of radioactivity was initially high in the liver, kidneys, and blood after the oral administration of [^{4}C] *d*-limonene *(Igimi et al., 1974); however, negligible amounts of radioactivity were found after 48 h. Differences between species regarding the renal disposition and protein binding of *d*-limonene have been observed. For rats, there is also a sex-related variation *(Lehman-McKeeman et al., 1989; Webb et al., 1989). The concentration of *d*-limonene equivalents was about 3 times higher in male rats than in females, and about 40% was reversibly bound to the male rat specific protein, $\alpha 2\mu$-globulin *(Lehman-McKeeman et al., 1989; *Lehman-McKeeman & Caudill, 1992).

The biotransformation of *d*-limonene has been, studied in many species, with several possible pathways of metabolism (Figure 1). Metabolic differences between species have been observed with respect to the metabolites present in both plasma and urine. About 25-30% of an oral dose of *d*-limonene in humans was found in urine as *d*-limonene-8,9-diol and its glucuronide; about 7-11% was eliminated as perillic acid (4-(1-methylethenyl)-1-cyclohexene-l-carboxylic acid) and its metabolites; *(Smith et al., 1969; *Kodama et al., 1976). *d*-Limonene-8,9-diol is probably formed via *d*-limonene-8,9-epoxide *(Kodama et al., 1976; *Watabe et al., 1981). In another study, perillic acid was reported to be the principal metabolite in plasma in both rats and humans *(Crowell et al., 1992). Other reported pathways of limonene metabolism involve ring hydroxylation and oxidation of the methyl group *(Kodama et al., 1976).

Following the inhalation exposure of volunteers to *d*-limonene at 450 mg/m^3 (79.6 ppm) for 2 h, three phases of elimination were observed in the blood, with half-lives of about 3, 33, and 750 min, respectively *(Falk-Filipsson et al., 1993). About 1% of the amount taken up was eliminated unchanged in exhaled air, whereas about 0.003% was eliminated unchanged in the urine. When male volunteers were administered (per os) 1.6 g [^{14}C] *d*-limonene, 50-80% of the radioactivity was eliminated in the urine within 2 days *(Kodama et al., 1976). Limonene has been detected, but not quantified, in breast milk of non-occupationally exposed mothers *(Pellizzari et al., 1982).

Five major plasma metabolites, perillic acid (major in most patients), dihydroperillic acid, limonene-1,2-diol, a previously undescribed analog of perillic acid and limonene-8,9-diol were identified in patients with advanced cancer following oral therapy with limonene in 21-day cycles (0.5 to 12 g/m^2/d) (Vigushin et al., 1998). Limonene circulated at levels similar to those of the metabolites. No accumulation of the metabolites was found after repetitive dosing for 21 days. The major urinary metabolites were glucuronide conjugates of perillic acid, dihydroperillic acid, limonene-8,9-diol and a monohydroxylimonene.

Figure 3 – Possible metabolic pathways of d-limonene



From Kodama *et al.* (1976)

M-I, *p*-Mentha-1,8-dien-10-ol; M-II, *p*-menth-1-ene-8,9-diol; M-IV, perillic acid-8,9-diol; M-V, *p*-mentha-1,8-dien-10-yl-β-D-glucopyranosiduronic acid; M-VI, 8-hydroxy-*p*-menth-1-en-9-yl-β-D-glucopyranosiduronic acid; M-VII, 2-hydroxy-*p*-menth-8-en-7-oic acid; M-VIII, perillylglycine; M-IX, perillyl-β-D-glucopyranosiduronic acid; M-X, *p*-mentha-1,8-dien-6-ol; M-XI, *p*-menth-1-ene-6,8,9-triol

10. Effects on Laboratory Mammals and In Vitro Test Systems

10.1 Single exposure

The acute toxicity of *d*-limonene in rodents is fairly low after oral, intraperitoneal, subcutaneous, and intravenous administration, based on the magnitude of the LD50 values (Table 10.1). LD50 values were approximately 5 g/kg bw for the oral administration of *d*-limonene or *d/l*-limonene to rats and for dermal application of *d/l*-limonene to rabbits and 6 g/kg bw for oral administration to mice *(Tsuji et al., 1974; *Tsuji et al., 1975b; *Opdyke, 1978). Studies on the acute inhalation toxicity of limonene were not identified.

Table 10.1 - Acute toxicity of limonene

Species (sex)	Route of administration	Type of limonene	LD50 (g/kg bw)	Reference
rabbit	dermal	d/l	> 5	*(Opdyke, 1978)
rat	oral	d/l	5.3	*(Opdyke, 1978)
rat (m/f)	oral	d	4.4/5.1	*(Tsuji et al., 1975b)
rat (m/f)	intraperitoneal	d	3.6/4.5	*(Tsuji et al., 1975b)
rat (m/f)	intraperitoneal, 3 d	d	0.125/0.11	*(Tsuji et al., 1975b)
mouse (m/f)	oral	d	5.6/6.6	*(Tsuji et al., 1975b)
mouse (m/f)	oral, 7 d	d	5.3/6.8[a]	*(Tsuji et al., 1974)
mouse (m/f)	intraperitoneal, 3 d	d	3.1/3.0[a]	*(Tsuji et al., 1974)
mouse (m + f)	intraperitoneal	d	1.3	*(Tsuji et al., 1975b)
mouse (m/f)	intraperitoneal, 10 d	d	0.59/0.50[a]	*(Tsuji et al., 1974)
mouse (m + f)	subcutaneous	d	> 41.5	*(Tsuji et al., 1975b)
mouse (m + f)	subcutaneous, 7 d	d	> 21.5	*(Tsuji et al., 1974)

[a] Calculated from ml/kg body weight.

Effects observed following the acute exposure of rodents to limonene include increased bile flow at 85 mg/kg bw *(Kodama et al., 1976), inhibition of S-3-hydroxy-3-methylglutaryl-CoA reductase activity at 409 mg/kg bw *(Clegg et al., 1980), enzyme induction at 600 and 1200 mg/kg bw *(Ariyoshi et al., 1975), and decreased motor activity, hypothermia and potentiation of hexobarbital-induced sleep at 3 ml/kg bw *(Tsuji et al., 1974).

10.2 Irritation and sensitisation

d-Limonene is considered a skin irritant *(Cronin, 1980; *Fischer, 1986). The skin irritancy of limonene in guinea-pigs *(Klecak et al., 1977) and rabbits *(Lacy et al., 1987; *Okabe et al., 1990) is considered moderate and low, respectively. In an in vivo study of rabbit skin irritation, *d*-limonene was ranked 3.5 of 8 on the basis of the primary irritation index *(Bagley et al., 1996); effects were graded according to OECD Test Guideline 404 *(OECD, 1981c). In a study in rabbits, *d*-limonene caused irritation to the eyes *(Tsuji et al., 1974).

Airborne *d*- and *l*- limonene produced sensory irritation in mice over a 30 min period (Larsen et al., 2000). The estimated NOEL was 100 ppm (556 mg/m^3), and the *d*- isomer was considered more potent. The RD50 values (exposure concentration decreasing respiratory rate by 50%) of *d*-limonene was estimated to be 1076 ppm (5983 mg/m^3) and for *l*-limonene 1467 ppm (8156 mg/m^3), based on the first 10 min of the exposure period. Pulmonary irritation and general anaesthetic effects were absent below 1600 ppm (8896 mg/m^3). Mild bronchoconstriction was seen above 1000 ppm (5560 mg/m^3). An earlier study (Kasanen et al., 1999) estimated the RD50 of limonene enantiomers to be between 1279 ppm and 4663 ppm (7111 mg/m^3 and 25 926 mg/m^3), based on testing of other terpenes and mixtures.

Although *d*-limonene was once considered the main allergen in citrus fruits, data from more recent studies in animals have revealed air-oxidized *d*-limonene, rather than unoxidized *d*-limonene, to be the sensitising agent. When limonene (unspecified form and unknown purity of the test material) was tested in four different sensitisation assays with guinea-pigs (Open Epicutaneous Test, Maximization Test, Draize's Test, and a test with Freund's Complete Adjuvant), it was sensitising in all but Draize's Test *(Klecak et al., 1977). In another study in mice, *d*-limonene did not induce sensitisation *(Maisey & Miller, 1986). Hydroperoxides and other oxidation products of *d*-limonene formed on exposure to the air have proved to be potent contact allergens when tested with Freund's Complete Adjuvant in guinea-pigs, whereas unoxidized *d*-limonene did not cause any sensitisation (Karlberg et al., 1991; Karlberg et al., 1992).

It has been suggested that limonene has a quenching effect (inhibition of the sensitising capacity of another substance) on the induction of skin sensitisation to citral. No effect was found in trials on guinea pigs or in the murine local lymph node assay (LLNA) (European Union, 2000).

10.3 Short-term exposure

Increases in hepatic cytochrome P-450 content have been observed in female rats administered limonene (isomer unspecified; 40 mg/kg bw/d for 3 days) by intraperitoneal injection *(Austin et al., 1988) and in rats administered 5% *d*-limonene in the diet for 2 weeks *(Maltzman et al., 1991). Increased epoxide hydratase activity was observed in rats administered 1% or 5% *d*-limonene in the diet for 2 weeks *(Maltzman et al., 1991). Increases in phase II enzymes (glutathionyltransferase and UDP-glucuronyltransferase) during the exposure of rats to 5% limonene in food have also been described *(Maltzman, 1991). Increased relative liver weight (from 5 to 20 times) has been observed in rats administered *d*-limonene at a dose of 75-300 mg/kg bw, at 300 mg/kg bw, the increase was significant *(Kanerva et al., 1987). In cats, infusion of 97% *d*-limonene into the bile system to dissolve gallstones caused acute and chronic inflammatory changes *(Schenk et al., 1980).

10.4 Long-term exposure

10.4.1 Subchronic exposure

Peroral administration of *d*-limonene to rats at a dose of 400 mg/kg bw for 30 d resulted in a 20-30% increase in the amount and activity of different liver enzymes (cytochrome P-450, cytochrome b5, aminopyrine demethylase, and aniline hydroxylase), increased relative liver weight, and decreased cholesterol levels *(Ariyoshi et al., 1975). Administration of *d*-limonene (0, 2, 5, 10, 30, and 75 mg/kg bw/d) by gavage to groups of 10 male rats, 5 d/week for 13 weeks *(Webb et al., 1989), resulted in the pathological formation of granular casts at the outer zone of the renal medulla. The no-observed-effect level (NOEL), based upon histological examination of the kidneys, was considered to be 5 mg/kg bw/d. The LOEL for increased liver and kidney weight was 75 mg/kg bw/d, the highest dose tested. The NOEL for effects in the liver was 10 mg/kg bw; the no-observed-adverse-effect level (NOAEL) for effects in the liver was 30 mg/kg bw/d. Linear regression analysis revealed a dose-related trend in the increased relative weights of the kidney and liver at 30 and 75 mg/kg bw/d. No histopathological changes were observed in the liver in these two studies. The amount and activity of different liver enzymes were not investigated, and thus the increase in relative liver weight may be due to enzyme induction. It is considered that the observed effects in the liver are probably signs of a physiological adaptation (Karlberg & Lindell, 1993).

10.4.2 Chronic exposure and carcinogenicity

The oral administration of *d*-limonene (0.4, 1.2, or 3.6 ml/kg bw/d) to dogs for 6 months caused nausea and vomiting *(Tsuji et al., 1975a). A 35% increase in alkaline phosphatase and cholesterol in serum and slightly increased total and relative liver weights were observed in dogs after peroral administration of *d*-limonene at a dose of 1.2 ml/kg bw/d for 6 months (about 1000 mg/kg bw/d) *(Webb et al., 1990).

In a 2-y study, *d*-limonene was administered (per os) 5 d/week to groups of 50 F344/N rats (0, 75, or 150 mg/kg bw/d to males, and 0, 300, or 600 mg/kg bw/d to females) and B6C3F mice (0, 250, or 500 mg/kg bw/d to males, and 0, 500, or 1000 mg/kg bw/d to females) (NTP, 1990). Slightly lower body weights were observed for rats in the high-dose groups and female mice in the high-dose group; however, no clinical symptoms could be related to the administration of *d*-limonene. For female rats in the high-dose group, survival was reduced after 39 weeks (NTP, 1990). There was clear evidence of carcinogenic activity of *d*-limonene in male rats, based upon a dose-related increase in the incidence of hyperplasia and adenoma/ adenocarcinoma in renal tubular cells. However, there was no evidence of carcinogenicity in female rats or in male and female mice. The carcinogenic response in the kidney of male rats has been linked to a unique renal perturbation involving $\alpha 2\mu$-globulin.

To determine whether *d*-limonene would cause a sustained increase in renal cell proliferation and exhibit promoting activity for the development of renal adenomas in male F344 rats, the animals were administered (by stomach tube) *d*-limonene (150 mg/kg bw/d) as a promoter 5 d/week for 30 weeks *(Dietrich & Swenberg, 1991). N-ethyl-N-hydroxyethyl-nitrosamine (500 ppm) (2780 mg/m^3) (was used as an initiator in the drinking-water for 2 weeks. In addition, male $\alpha 2\mu$--

globulin-deficient rats were exposed in the same manner to determine if the mate rat specific urinary protein α2μ-globulin is required for *d*-limonene to cause these effects. Exposure to *d*-limonene alone caused a significant increase in the number of atypical tubules and atypical hyperplasias in F344 rats, compared with vehicle controls. There was no increase in the incidence of tumours or preneoplastic lesions in the α2μ-globulin deficient rats exposed to *d*-limonene, whereas a 10-fold increase in the incidence of renal adenoma and atypical hyperplasia was observed in F344 rats exposed to *d*-limonene compared with controls. There was a significant decrease in the incidence of liver tumours in animals exposed to N-ethyl-N-hydroxyethylnitrosamine and *d*-limonene, compared with N-ethyl-N-hydroxyethylnitrosamine exposure alone.

10.5 Genotoxicity and related end-points

On the basis of available data, there is no evidence that *d*-limonene or its metabolites are genotoxic or mutagenic. Limonene and its epoxides were not mutagenic when tested at concentrations of 0.3 to 3333 μg/plate in in vitro assays using different strains of Salmonella typhimurium, in the presence or absence of metabolic activation *(Florin et al., 1980; *Watabe et al., 1981; *Haworth et al., 1983; *Connor et al., 1985; NTP, 1990). *d*-Limonene did not increase the frequency of forward mutation at the TK+/- locus in mouse L5 178Y cells (NTP, 1990), induce cytogenetic damage in Chinese hamster ovary cells *(Anderson et al., 1990), or malignantly transform Syrian hamster embryo cells *(Pienta, 1980). In one in vitro study, following exposure with benzo(a)pyrene, *d*-limonene (21.9 μmol/litre) inhibited the formation of transformed cell colonies in tracheal epithelium isolated from rats *(Steele et al., 1990).

No evidence of mutagenicity was reported in an in vivo spot test with mice, involving the intraperitoneal injection of limonene at 215 mg/kg bw/d on days 9-11 during gestation *(Fahrig, 1984).

10.6 Reproductive and developmental toxicity

Studies on the reproductive toxicity of limonene were not identified. There is no evidence that limonene has teratogenic or embryotoxic effects in the absence of maternal toxicity. In rats, the oral administration of *d*-limonene (2869 mg/kg bw/d) on days 9-15 of gestation resulted in decreased bw and deaths among the dams. Delayed ossification and decreased total body and organ weights (thymus, spleen, and ovary) were observed in the offspring *(Tsuji et al., 1975b). In mice, the oral administration of *d*-limonene (2869 mg/kg bw/d) on days 7-12 of gestation resulted in reduced growth in the mothers and a significantly increased incidence of skeletal anomalies and delayed ossification in the offspring *(Kodama et al., 1977a). The oral administration of *d*-limonene (250, 500, or 1000 mg/kg bw/d) to rabbits on days 6-18 of gestation had no dose-related effects on the offspring. At the highest dose, there were some deaths and reduced weight gain among the dams; at the intermediate dose, growth was decreased *(Kodama et al., 1977b).

10.7 Immunological and neurological effects

Reports relating limonene to type I allergy were not identified. In a study designed to assess the immunological effects of *d*-limonene on B- and T-cell responses, BALB/c mice were administered (by forced intragastric feeding) *d*-limonene (0.1

ml) daily for 9 weeks *(Evans et al., 1987). Mice given keyhole limpet haemocyanin prior to exposure to *d*-limonene had suppressed primary and secondary anti-keyhole limpet haemocyanin responses. Mice exposed to *d*-limonene prior to the administration of keyhole limpet haemocyanin had significantly increased antibody and mitogen-induced proliferative responses. However, the purity of the *d*-limonene in this study was not checked, and oxidation products may have been the active substances.

Effects on the central nervous system following exposure to limonene have been reported in experimental studies with animals; however, it is difficult to ascertain whether these effects were the result of general intoxication or a more direct effect of the chemical. The peroral administration of *d*-limonene (3 ml) to rats and mice resulted in decreased motor activity *(Tsuji et al., 1974). A similar effect was also observed in mice orally administered a limonene dose of 1000 mg/kg bw/d for 13 weeks (NTP, 1990).

10.8 Therapeutic use

d-Limonene has chemopreventive activity against rodent mammary, skin, liver, lung and forestomach cancers when fed during the initiation phase (Crowell, 1999). *d*-Limonene and its metabolite perillyl alcohol have chemotherapeutic activity against rodent mammary and pancreatic tumours and this property is under evaluation in Phase I clinical trials. Multiple mechanisms are believed to be responsible for the anti-carcinogenic effects.

11. Effects on Humans

Case reports or epidemiological studies on the effects of limonene on human health were not identified except for sensitisation and irritation. Available data on other effects have been derived from studies with volunteers. In older investigations, multiple exposures and confounding factors such as mechanical damage, irritation, other allergens, and infections due to wet work *(Beerman et al., 1938; *Schwartz, 1938; *Birmingham et al., 1951) may have contributed to the effects reported following exposure to limonene. None of eight subjects reported any discomfort, irritation, or symptoms related to central, nervous system effects during a 2-hour inhalation exposure to *d*-limonene at 10, 225, or 450 mg/m^3 (1.7, 39.8, or 79.6 ppm); however, a slight decline in vital capacity was observed following exposure to the highest concentration *(Falk-Filipsson et al., 1993).

Eye irritation of *l*-limonene was measured using goggles instrumentation and a relatively short exposure time of 2 minutes. The threshold level for irritation in 12 volunteers was 1700-3400 mg/m^3 (300-600 ppm approximately) (Mølhave et al., 2000).

Oxidised Histo-clear solvent, identified elsewhere as limonene-based (Langman, 1995), produced respiratory irritation requiring medical treatment in a histology technician (Zitko, 2001). It was considered that the autoxidation products might be the cause of the irritation. An increase in nasal or throat irritation was also reported by 4/12 workers in a facility where a limonene-based cleaner was stored in an uncovered tank (Kiefer et al., 1994).

In a study in which the sensitivity of four patch testing systems (Finn chamber, Hill Top patch, Van der Bend chamber, and Webril patch) was evaluated in volunteers, *d*-limonene (perfume-grade) reacted strongly in all types of patches within 10-15 minutes of exposure *(York et al., 1995). Skin irritation was assessed before application, as well as immediately and 1, 24, 48, and 72 hours after removal of the patch, using a scoring system based broadly on that used for rabbit irritation studies *(OECD, 1981c), but modified to account for the nature of reactions on human skin. There was evidence of sensory effects and urticarial responses on removal of the patches. Significant irritation persisted for 24 hours, and these reactions persisted for 48 and 72 hours in many volunteers *(York et al., 1995). Dermal exposure to *d*-limonene (98%) for 2 hours in one subject caused burning, itching, aching, and a long-lasting purpuric rash *(Falk et al., 1991).

d-Limonene infused directly into the bile system of human volunteers to dissolve gallstones caused pain in the upper abdomen, nausea, vomiting, and diarrhoea, as well as increases in serum aminotransferases and alkaline phosphatase *(Igimi et al., 1976, *Igimi et al., 1991). The oral administration of 20 g *d*-limonene to volunteers resulted in diarrhoea, painful constrictions, and proteinuria, but no biochemical changes (total protein, bilirubin, cholesterol, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase) in the liver *(Igimi et al., 1976). Dose related gastrointestinal side effects such as nausea and diarrhoea were observed following oral administration of *d*-limonene to patients with advanced cancer in a Phase I clinical trial. Dosages were 0.5 to 12 g/m^2 body surface area (BSA) per day and the maximum tolerated dose was 8 g/m^2 BSA

(Vigushin et al., 1998). The authors reported that *d*-limonene had low toxicity after single and repeated dosing for up to 1 y.

There are no studies involving inhalation exposure in which effects on the liver have been investigated.

Reports of contact allergy to dipentene have appeared *(Calnan, 1979; *Rycroft, 1980). In one investigation, 15 of 22 people with an allergy to oil of turpentine also reacted to dipentene *(Cachao et al., 1986). Patch testing in consecutive dermatitis patients from Sweden and Belgium revealed positive reactions in 1.5-2% of the subjects tested with oxidized *d*-limonene, a finding similar to that observed with other common sensitisers, such as formaldehyde (A.T. Karlberg, personal communication, 1996; Karlberg & Dooms-Goossens, 1997). The oxidation products *d*-cis- and *d*-trans-limonene-1,2-oxide, carvone, and cis- and trans-limonene-2-hydroperoxide were found to be sensitisers. Some patients not sensitive to the oxidised limonene were found to be sensitive to the hydroperoxide fraction, and vice versa. It is believed that limonene itself has a very low sensitising capacity and that sensitisation is due to the oxidation products formed on exposure to air.

No sensitising effect was observed when 25 volunteers were exposed to *d*-limonene in a Human Maximization Test *(Grief, 1967). Skin patch testing carried out at an Australian dermatology practice in 1999 indicated that 2/470 patients reacted to limonene, and a larger number reacted to other terpenes. The oxidation status of the limonene was not tested (Fewings, 2001).

Prolonged or repeated exposure to limonene in histology laboratories is reported to cause respiratory effects such as difficulty in breathing and tightness in the chest (Dapson & Dapson, 1995). Bronchial hyper-responsiveness was related to indoor concentrations of limonene in dwellings (Norbäck et al., 1995).

Health effects reported in the NICNAS phone survey of formulators and users included skin drying and irritation, dermatitis, eye irritation, headache and “lightheadedness” from the strong odour, and respiratory symptoms when product is atomised (see Appendix 3).

d-Limonene reduced non-immunological contact urticaria caused by cinnamic aldehyde, with competitive receptor inhibition suggested as the mechanism of suppression *(Guin et al., 1984). Quenching (inhibition of the sensitising capacity of another substance) of the effects of citral by limonene has been reported in humans (European Union, 2000). However the EU SCCNFP noted conflicting evidence and concluded on the balance of evidence currently available that the quenching of fragrance allergens by other specific fragrance components should be regarded as a hypothesis only.

12. Environment Effects

No ecotoxicity tests were provided during the assessment. The following toxicity results have been obtained from the Concise International Chemical Assessment Document (IPCS, 1998) on limonene. Test reports and figures are stated as reported and have not been sighted. No chronic testing on any trophic level appears to have been conducted.

12.1 Aquatic environment

The acute toxicity of *d*-limonene ranges from slight to high for aquatic organisms. The lowest acute toxicity values (EC50 or LC50) identified were approximately 0.4 mg/L for daphnia and 0.7 mg/L for fish. The no-observed-effect concentration (NOEC) for green algae is approximately 4 mg/L. The acute toxicity (EC50 or LC50) of dipentene to daphnia and fish is about 50-70 times lower than that for *d*-limonene. Results of ecotoxicity testing of aquatic organisms are summarised below.

12.1.1 Toxicity to fish

Table 12.1 - Toxicity to fish (IPCS, 1998)

Species	Test Isomer	Test Duration	Test Conditions	Result (mg/L)
Fathead minnow (*Pimephales promelas*)	*d*-Limonene	96 h	Flow-through	LC50 = 0.702 (0.619-0.796)
				LC50 = 0.720 (0.618-0.839)
				LC50 = 0.688 (0.606-0.782)
	dipentene	96 h	Flow-through	LC50 = 38.5 (35.4-41.8)
				LC50 = 28.2
Fish	n.s.	n.s.	n.s.	LC50 = 80
	d-Limonene	n.s.	Flow-through	LC50 = 0.711
Golden orfe (*Leuciscus idus*)	dipentene	48 hours	n.s.	LC50 = 32

The results of testing indicate that *d*-limonene can be considered highly toxic while the racemic mixture *dl*-limonene is slightly toxic to fish under acute exposure.

12.1.2 Toxicity to aquatic invertebrates

Table 12.2 - Toxicity to aquatic invertebrates

Species	Test Isomer	Test Duration	Test Conditions	Result (mg/L)	Reference
Water flea (*Daphnia magna*)	*d*-Limonene	48 h	Flow-through	LC50 = 0.577 (0.496-0.672)	IPCS, 1998
				EC50 = 0.421	
	n.s.	n.s.	n.s	LC50 = 39	
	dipentene	48 h	Flow-through	LC50 = 31 (27.5-34.8)	
				EC50 = 28.2	
Water flea (*Daphnia pulex*)	*d*-Limonene	48 h	Flow-through	LC50 = 0.730	
	n.s.	48 h	Static	LC50 = 69.6	
Daphnia	*d*-Limonene	21 d	Structure-activity relationship (SAR) analysis	NOEC = 0.15	
Mosquito fly (*Culex quinquesfasciatus*)	n.s.	72 h	2nd-instar (23-33°C), static	6.6-26.1	
			4th-instar (23-33°C), static	7.8-30.6	
Water hyacinth weevil *(Neochetina eichhorniae,* 60%, *N. bruchi,* 40%*)*	*d*-Limonene	n.s.	weevils dipped in limonene/ ethanol solutions	50% limonene 73% Mortality (range 40-100%)	Haag 1986
				10% limonene 13.3% Mortality (range 0-20%)	

The above results indicate that *d*-limonene can be considered highly toxic to aquatic invertebrates and the racemic mixture is slightly toxic. The racemate is also slightly toxic to mosquito fly.

12.1.3 Toxicity to aquatic plants

Table 12.3 - Toxicity to aquatic plants

Species	Test Isomer	Test Duration	Test Conditions	Result (mg/L)	Reference
Green algae	dipentene	96 h	Static	NOEC = 4.08	IPCS, 1998

Based on the results of this solitary test, the racemic mixture of limonene can be considered moderately toxic to aquatic plants.

12.2 Terrestrial environment

The toxicity of limonene has been studied in various terrestrial organisms (see Table 12.4). Limonene generally has moderate acute toxicity in insects and mites.

The acute toxicity of *d*-limonene to earthworms (*Eisenia foetida* Savigny) was high (LC50 = 6.0 ppm) (33.4 mg/m^3). Worms were exposed to limonene in capped 95 mL bottles containing filter paper (total surface area of paper = 115 cm^3) wetted with limonene. Sublethal effects (i.e. abnormal rebounding of medial giant fibre pathway [MGF] impulses and spontaneous lateral giant fibre pathway [LGF] spiking) were observed following exposure of earthworms to 4.2 ppm (23.3 mg/m^3) limonene (Karr et al., 1990).

Limonene has low subacute toxicity to bobwhite quail (*Colinus virginianus*) exposed via the diet (LC50 > 5620 ppm) (31 247 mg/m^3) (US EPA, 1994).

12.3 Summary of environmental effects

Following the guidelines from Mensink *et al* (1995), *d*-limonene can be described as highly toxic to aquatic vertebrates and aquatic invertebrates based on experimental acute exposure results. The racemic mixture is slightly to moderately toxic.

The limited results suggest invertebrates to be the most sensitive species in the aquatic system, with results for daphnia being 0.421 mg/L (48 h EC50).

12.4 Derivation of PNEC for aquatic organisms

d-Limonene

Since several results for fish and invertebrates are available, an assessment factor of 100 is applied to the lowest acute toxicity result, that is, the EC50 of 0.421 mg/L for daphnia. This gives a PNEC of 4.21 μg/L for *d*-limonene.

Dipentene

The data available for dipentene includes several results for fish and invertebrates and a chronic algal NOEC. Hence an assessment factor of 100 is applied to determine the PNEC from the lowest toxicity result (4.0 ppm for algae) (22.2 mg/m^3) giving a value of 40 μg/L for dipentene.

Table 12.4 – Toxicity to terrestrial organisms

Species	Test Isomer	Test Duration	Test Conditions	Result	Reference
Birds					
Bobwhite quail (*Colinus virginianus*)	n.s.	8 d	Acute Dietary Toxicity	LC50 > 5620 ppm	US EPA 1994
Insects					
Cat flea (*Ctenocephalides felis*)	*d*-Limonene	n.s.	Fleas exposed to filter papers treated with limonene, either directly or to vapours.	Adult; $LD50_{Contact}$ = 160 (157-163) µg/cm^2 Adult; $LD50_{vapour}$ = 259 (234-281) µg/cm^2 Pupae; $LD50_{Contact}$ = 376 (259-468) µg/cm^2 Larvae; $LD50_{Contact}$ = 226 (221-231) µg/cm^2 Eggs; lethal to all eggs on contact at 65 µg/cm^2	IPCS, 1998
Variegated cutworm (*Peridroma saucia)*	*d*-Limonene	n.s.	Larvae; significant inhibition of population; dietary exposure	0.2% limonene in artificial in feed	
German cockroach (*Blattella germanica* L.)	*d*-Limonene	24 h	Adult; Topical Fumigation	LD50 = 700 (610-810) µg/insect LC50 = 23.3 (17.5-31.0) ppm	
		n.s.	Adult and nymph; oral 25% limonene in feed	No mortality	
		72 h	Adult; contact with treated surface	No mortality (limonene concentration not given)	
		n.s.	Effect on growth rate; 1-25% limonene in diet Oothecae yielding young; topical exposure Effect on reproduction; 25% limonene in diet	Result not given EC50 = 0.68 mg/ootheca No effect	
Rice weevil (*Sitophilus oryzae* L.)	*d*-Limonene	24 h	Fumigation	19.0 (13.2-27.3) ppm	
House fly (*Musca domestica* L.)	*d*-Limonene	25 h	Topical	LD50 = 90 (70-130) µg/insect	
Western corn rootworm (*Diabrotica virgifera virgifera* LeConte)	*d*-Limonene	72 h	Egg; contact with treated substrate Larvae; contact with treated soil	LC50 = 1.8 (0.8-2.9) % limonene LC50 = 12.2 (4.5-32.6) ppm	
Spiders and allies					
Spruce spider mite (*Oligonychus ununguis* (Jacobi))	*l*-Limonene	24 h	Vapour	LC50 = 24.5 ppm A significant decrease in oviposition was observed at 5 ppm	IPCS, 1998
Segmented worms					
Earthworm (*Eisenia foetida* Savingy)	*d*-Limonene	48 h	n.s.	LC50 6.0 (5.1-7.1) ppm sublethal effects were observed at 4.2 ppm	IPCS, 1998

13. Risk Characterisation

13.1 Environmental risk

Limonene is a volatile chemical with low water solubility. It has a range of uses in Australia, being used as a solvent, as a flavour in food, as a fragrance and in a host of cleaning products. Release is expected to be predominantly to the atmosphere although exposure to aquatic systems may also result.

13.1.1 Aquatic compartment

The major release of limonene to the aquatic compartment is expected to be through its use in cleaning compounds. The PEC for both *d*-limonene and dipentene were determined in Section 8.1.3. The PEC/PNEC ratios show that *d*-limonene should not cause adverse effects on the aquatic compartment.

Table 13.1 - PEC/PNEC ratios determined for d-limonene and dipentene in the aquatic compartment

Isomer	$PEC_{effluent}$ (µg/L)	PEC/ PNEC	PEC_{water} (µg/L)		PEC/PNEC	
			High Dilution	Low Dilution	High Dilution	Low Dilution
d-limonene	8.9	2.1	0.51	2.5	0.12	0.61
dipentene	0.68	0.02	0.04	0.19	0.009	0.047

These values (worst case scenario) indicate that there will be no adverse effects on aquatic organisms arising from the formulation or end use of *d*-limonene or dipentene in Australia (see Section 8.1.3). This conclusion is in agreement with those outlined in the Concise International Chemical Assessment Document on limonene which concluded that because concentrations of limonene in surface waters of "polluted" and "unpolluted" areas are at least about 250 and 20 000 times lower than this acute toxicity value (0.4 mg/L; 48-h EC50 for daphnia), respectively, it is likely that limonene poses a low risk for acute toxic effects on aquatic organisms (IPCS 1998). No studies were identified on chronic effects, and therefore risks associated with chronic exposures of aquatic organisms to limonene in "polluted" waters cannot be determined.

13.1.2 Terrestrial compartment

Terrestrial organisms are most likely to be exposed to limonene via the air. The few studies on terrestrial species (i.e. insects) using vapour exposure reveal effects of limonene at parts per million levels. Measured environmental concentrations from overseas are typically around 0. 1 to 2 ppb (0.6 to 11 $\mu g/m^3$), indicating a low risk for acute toxic effects on terrestrial organisms from direct exposure to limonene in air. At polluted sites, limonene concentrations in soil (up to 920 mg/kg soil in Florida) may exceed effect levels of soil-living organisms (e.g. earthworm, acute LC50 = 6.0 ppm) (33.36 mg/m^3).

13.1.3 Atmosphere

Limonene and other terpenes are released in large amounts mainly to the atmosphere. When released to soil or water, limonene is expected to evaporate to air to a significant extent, owing to its high volatility. Thus, the atmosphere is the predominant environmental sink of limonene, where it is expected to rapidly undergo gas-phase reactions with photochemically produced hydroxyl radicals, ozone, and nitrate radicals. The oxidation of terpenes, such as limonene, contributes to aerosol and photochemical smog formation. Ozonolysis of limonene may also lead to the formation of hydrogen peroxide and organic peroxides, which have various toxic effects on plant cells and may be part of the damage to forests observed in the last decades. Emissions of biogenic hydrocarbons such as limonene and other terpenes to the atmosphere may either decrease ozone concentrations when oxides of nitrogen concentrations are low or, if emissions take place in polluted air (i.e. containing high oxides of nitrogen levels), lead to an increase in ozone concentrations (IPCS 1998). Limonene has not been identified as an air toxic in Australia and is not on the list of substances reported to the National Pollutant Inventory.

13.2 Occupational risks

In this section, the results of the hazard and occupational exposure assessments have been combined to characterise the potential risks of adverse health effects in workers exposed to limonene.

Results from risk characterisation provide the basis for risk management strategies to reduce exposure and increase worker awareness of potential hazards and safe handling of limonene.

13.2.1 Critical health effects

Limonene is a skin irritant. It has the potential to cause eye irritation and respiratory irritation, but data on these effects are limited. Acute toxicity is low.

Limonene is not a skin sensitiser, but its oxidation products have the potential to be skin sensitisers. Limited data are available on the potential to cause respiratory sensitisation.

Renal tumours and related effects on the kidney found in male rats are sex and species specific and are not considered to be relevant to humans. Other than in male rats, the critical organ in animals is the liver, with effects having been observed in mice, rats and dogs. Exposure affects the amount and activity of liver enzymes, liver weight, cholesterol levels and bile flow. However, no microscopic changes have been reported in the liver at these or higher levels. The liver effects in animals are probably due to physiological adaptation. In addition, effects of inhalational exposure on the liver have not been investigated in humans or animals. From the data available, it is not possible to set a NOAEL for liver effects.

13.2.2 Risk estimates

Risks from physicochemical hazards

Limonene is a flammable liquid with a flash point of 48^{0}C. Its flammability limits in air are 0.7% to 6.1% by volume. Explosive vapour/air mixtures may be formed at temperatures above 48°C. It may decompose to produce toxic fumes of carbon monoxide. Rags or other combustible material that have been wet or soaked with limonene may spontaneously combust.

A potential fire/explosion risk exists for limonene during manufacture, transport, storage and end use. Distillation to dryness may also lead to concentration of peroxides and the risk of explosion. Distillation during manufacture occurs, however the fresh material is expected to be low in peroxides. Distilling limonene in order to recycle it was not reported, but would be expected to have a higher risk. Information provided by industry indicated that the material is manufactured in an enclosed system and is transported and stored according to the regulations for Class 3 flammable goods after importation. The fire/explosion risk, therefore, is significantly reduced. However, it is of concern when limonene is exposed to heat, static spark, open flame or other ignition sources during repacking, formulation and end use.

Risks from manufacture, importation, repacking and use of limonenes

Acute risks

The risk of skin, eye and respiratory irritation during manufacture and importation of limonene is minimal as potential exposure to the chemicals is unlikely or very low, except in cases of accidental spills or leaks of the chemicals.

The risk of acute effects during repacking and formulation of limonene products is expected to be low due to periodic rather than daily exposure and use of PPE at all sites. In addition, local exhaust ventilation at some workplaces further reduces risk. Nevertheless, there is the possibility of spillage during repacking and formulation as manual handling procedures are employed at some plants during some stages of formulation such as transfer of raw material to another container or the mixing vessel, during mixing and handling of final products. Consequently, there is a potential for skin, eye and respiratory irritation during clean-up of spills. Cleaning of the storage tank for repacking (see Section 8.2.5) is of concern as workers are exposed to high concentrations of limonene in a confined space.

Although limonene has a low vapour pressure, the rate of vaporisation of limonene is high. Manual handling of limonene and limonene products occurred during the majority of the identified end uses. Therefore, there may be a risk of skin, eye and respiratory irritation for end users from acute exposure to limonene and/or products containing limonene following direct skin contact and contact with airborne particles or secondary transfer from hands or gloves. The risk is expected to be higher in the following situations:

- use of raw material or products containing high percentages of limonene;
- use in confined spaces and in places with limited ventilation;
- application of products by spraying or during hosing off of the products;

- where PPE is not used.

However, in workplaces where limonene or limonene products are used in an enclosed (automated) system or in open spaces with good natural ventilation, the risk of acute effects is expected to be low.

In addition to normal usage, acute exposure of end users to limonene may occur following accidental spillage. As the package sizes of limonene and limonene products vary largely and can be as large as 200 L drums, exposure could be significant especially via inhalation. However, this is likely to be an uncommon situation.

Risks from repeated exposure

There are no Australian or overseas measured exposure data available for manufacture, importation, repacking and formulation of limonene/limonene products. Limited overseas monitoring data are available for a number of end use scenarios. Although exposure levels during manufacture and formulation were estimated using the EASE model, the margin of exposure (MOE) approach for risk characterisation was not undertaken, as an appropriate NOAEL was not identified for effects on the liver in animals.

Potential exposure to limonene during manufacture is limited due to manufacture occurring in enclosed systems. Exposure may occur during sampling of *d*-limonene, but this is done only periodically and is of short duration. Dermal exposure to limonene using the EASE model was estimated to be very low and inhalational exposure was estimated as 0 to 0.1 ppm (0 to 0.56 mg/m^3). Therefore, the risk of developing any adverse effects during manufacture is very low.

Risk of health effects during importation is minimal as occupational exposure during importation, transportation and storage is unlikely except in cases of accidental spills or leaks of the chemicals.

For workers involved in repacking activities, the risk is expected to be low as in the majority of the workplaces repacking is done periodically and the whole process including cleaning of equipment is of short duration. In addition, information provided by industry indicates that PPE is used at all plants where repacking occurs and LEV is used at some of the workplaces. The risk would be minimal for workers who repack products containing a low concentration of limonene and where the repacking process is enclosed.

During formulation of limonene products, dermal contact is likely during manual charging of mixing vessels, mixing and inspection, filling of product containers and cleaning equipment. There is also a potential for inhalation exposure, especially during heated blending process and high-speed mechanical stirring. Risk to workers during formulation is likely to be higher at worksites with an open mixing process and no exhaust ventilation. However, according to the data provided for assessment, formulation is a batch process and potential for exposure to limonene occurs only on the days when limonene based products are formulated. The adjusted EASE model estimations for formulation are low. The model estimated levels of up to 0.25 ppm (1.4 mg/m^3) with LEV and 1.25 ppm (7 mg/m^3) without LEV at workplaces. Dermal exposure is estimated to be as low as 0.1 $mg/cm^2/d$. Therefore, it is reasonable to assume that the risk of health effects from repeated exposure during formulation is low.

Potential for exposure during end use of limonene or limonene products is high due to the widespread uses, the modes of use and lack of control measures at some worksites. Overseas monitoring data are available for some work scenarios. Personal monitoring of workers involved in graffiti removal over 8 h measured levels of up to 0.52 mg/m^3 (0.09 ppm). Aerosol formation is likely during graffiti removal as the product is sprayed on and removed either by wiping or hosing. The monitoring data indicate that risk of adverse effects is likely to be low. Personal monitoring data over 8 h are also available during degreasing of aircraft parts. Levels as high as 634 mg/m^3 (112 ppm) were measured at the worksite. The engineering controls used at the workplace were not stated. Risk of adverse health effects during end use of limonene and limonene products is likely to be low, as no critical effects have been identified in humans following repeated exposure. However, a high risk exists when these chemicals are used in confined spaces and in places with limited ventilation. Monitoring data indicated levels as high as 3158 mg/m^3 (559 ppm) over 8 h during use of limonene products for metal stripping in enclosed spaces with limited ventilation. The concentration of limonene in the products was not reported.

The risks of both acute and chronic effects are likely to be high when limonene or limonene products are used in confined spaces. The minimum requirement for working in a confined space is set out in the Australian Standard AS 2865 *Safe Working in a Confined Space* (Standards Australia, 1995). In addition, states/territories may have legislative requirements to minimise exposure to hazardous chemicals when working in confined spaces.

Skin sensitisation of workers can occur if there is exposure to oxidised limonene. The sensitising properties of limonene are acknowledged to be due to its oxidation products. There is no threshold level for sensitising effects. Exposure can occur through inadvertent contact with e.g. cleaning products containing limonene or deliberate contact with industrial hand cleaners containing limonene. For the latter type of use, exposure cannot be controlled because it is an inherent part of the product's use.

Oxidation products of limonene form on storage, and the extent varies with the type of handling and the precautions taken against autoxidation. No information is available on the formation of oxidation products in mixtures. Key precautions against autoxidation are addition of antioxidant, protection against air, light and elevated temperature, and monitoring shelf life (Section 14.1).

Skin exposure can be minimised for only some of the uses of limonene, and prevention of autoxidation is critical to prevention of skin sensitisation. In hand cleaner use there is deliberate dermal contact, and some products can be wiped off rather than washed off, increasing the duration of exposure. Even for uses where skin contact is inadvertent, the risk of skin sensitisation exists because there is no threshold for sensitising effects. Risk of skin sensitisation is high in situations where oxidation products of limonene are likely to be formed.

13.2.3 Uncertainties in risk characterisation

Uncertainties in any risk characterisation process arise from inadequate information, assumptions made during the process and variability in experimental conditions. These uncertainties need to be considered when deciding if an estimated exposure is of concern. Examples of uncertainties inherent in the characterisation of risk for limonene arise mainly from inadequate data and include:

- limited data from NICNAS use and exposure surveys;
- lack of representative atmospheric monitoring;
- lack of data on the health effects of high doses limonene in humans following repeated exposures;
- no clear NOAEL identified from animal studies;
- lack of data on the permeability of limonene through the skin; and
- lack of data on autoxidation of limonene in mixtures.

In addition, the assumptions used in the EASE modelling add uncertainties to the risk characterisation.

13.2.4 Areas of concern

Risk characterisation has indicated that the risk of fire/explosion is of concern, especially when limonene is exposed to heat, static spark, open flame or other ignition sources.

There may be a risk of skin, eye and respiratory irritation when working in a confined space during cleaning of a storage tank for repacking and during end use of pure limonene and/or products containing high concentrations of limonene, especially when used in confined spaces and in places with limited ventilation, by certain application modes such as spraying or where PPE is not used.

Risk characterisation has indicated that the risk of health effects from repeated exposure during end use of limonene or limonene products, in confined spaces and in places with limited ventilation is of concern.

The risk of skin sensitisation exists in situations where oxidation products of limonene occur. This risk can be significantly reduced by minimising autoxidation of limonene. Control of oxidation is critical in hand cleaners containing limonene, where skin contact is deliberate. When "wipe-off" hand cleaners are used, the duration of skin contact is increased, with higher potential for sensitisation.

13.3 Public health risk

With regard to the potential for skin sensitisation, it is generally recognised that it is the oxidation products of limonene that posses the potential for skin sensitisation and not limonene itself. Limonene in consumer products can oxidise over time, thereby products with relatively high concentrations of limonene, used infrequently, would have long "shelf-lives" and could contain oxidation products that may pose a sensitisation hazard to sensitive individuals. This oxidation potential in consumer products could be removed by the incorporation of an anti-oxidant (e.g. 0.1-0.2% BHT (Karlberg et al., 1994)), as recommended in the IFRA guidelines "*d*-, *l*-, and *dl*-limonene and natural products containing it should only be used when the levels of peroxides is kept to the lowest practical level, for instance by adding anti-oxidants at the time of production. Such products should have a peroxide value of less than 20 mmols/L, determined according to the Fragrance Materials Association (FMA) method". Prevention of the formation of oxidation products of limonene would eliminate its sensitisation potential. Some consumer products may already contain ingredients with anti-oxidant properties for other reasons e.g. skin care benefits in dermally-applied products. In addition the oxidation potential of limonene in mixtures (all consumer products containing limonene have other ingredients) is unknown.

Allergies to citrus fruits, which may be due to oxidation products of limonene, are usually known from an early age (due to peeling and ingestion). Sufferers are likely to avoid consumer products containing limonene, as citrus components may be advertised on labelling and packaging, or may be detected by smell (limonene has a very low odour threshold). Consequently the risk of sensitisation is reduced by avoidance.

From the available information, public exposure will be widespread, but the hazards i.e. irritation and the potential for sensitisation to oxidised forms of limonene, are quite low.

14. Risk Management

The key elements in the management of health and safety risks from exposure to hazardous substances include

- control measures;
- hazard communication;
- atmospheric monitoring;
- regulatory controls; and
- emergency procedures.

An assessment of the measures currently employed and/or recommended to reduce health and environmental risks associated with the use of limonene and products containing it is included in this section. Basic information concerning the MSDS and labels supplied by the importers and formulators is also included.

14.1 Assessment of current occupational control measures

According to the NOHSC *National Model Regulations for the Control of Workplace Hazardous Substances* (NOHSC, 1994a), exposure to hazardous substances should be prevented, or where that is not practicable, controlled to minimise risks to health. NOHSC's *National Code of Practice for the Control of Workplace Hazardous Substances* (NOHSC, 1994b) lists the hierarchy of control measures, in priority order, that should be implemented to eliminate or minimise exposure to hazardous substances. These are:

- elimination;
- substitution;
- isolation;
- engineering controls;
- safe work practices; and
- personal protective equipment.

Elimination and substitution

Elimination is the removal of a chemical from a process and should be the first option considered when minimising risks to health. In situations where it is not feasible or practical to eliminate the use of a chemical, substitution should be considered. Substitution includes replacing with a less hazardous substance or the same substance in a less hazardous form.

Substitution or elimination of limonene/dipentene may not have been considered by many users or formulators, because it is itself considered to be a less hazardous replacement for other solvents. Over the last 10 years or so, many users have re-evaluated their use of solvents in order to replace those found to be ozone-depleting e.g. 1,1,1-trichloroethane or those of concern for toxicity e.g. trichloroethylene. Limonene is a terpene, one of the chemical groups that has been considered as a direct or indirect substitute for other solvents.

In the choice of a solvent, users need to evaluate the technical issues, cost, health and safety and environmental effects of each option when considering substitution. Examples of internet resources available to aid solvent substitution are the SAGE Solvent Alternatives system (Research Triangle Institute & U.S. EPA, 2001), guidance from USA State government authorities (Washington State Dept of Ecology, 1996) (Ohio EPA, 1997) (Oregon DEQ) and a North Atlantic Treaty Organization (NATO) paper (NATO, 1994).

General suggestions for solvent elimination include use of blast cleaning with dry ice (carbon dioxide) or other media, vacuum de-oiling, and measures that would minimise or remove the need to clean.

Where limonene is chosen as a solvent, exposure may be reduced by using lower concentrations (e.g. in semi-aqueous formulations).

Risks from the flammable characteristics of limonene may be reduced if mists are not formed e.g. through spraying (Urben, 1999) and if the material is kept below its flash point of 48^0C.

Minimisation of oxidation is a measure that would reduce the allergenic potential of limonene and thus its hazard. Precautions against oxidation can also reduce unwanted odour and colour changes that are commercially undesirable. Measures recommended or reported by suppliers and users include:

- addition of antioxidant; antioxidant is present in some imported material and is added by a minority of formulators. Some companies do not know whether the material they are using contains antioxidant, or use grades of limonene with and without antioxidant interchangeably. One local manufacturer reported that addition of anti-oxidant to limonene had not been successful in trials carried out by their company as darkening of the material occurred. The change in colour may have been a marker of oxidation;
- storage in air-tight containers;
- minimisation of head-space in drums. One reseller transfers the contents of partly used drums into smaller containers;
- purging head-space of drums with nitrogen or carbon dioxide;
- keeping away from light. This is reported as a general precaution against autoxidation;
- keeping material cool by controlled storage conditions. One supplier recommends storage out of sunlight (preferably indoors) and at < 35^0C, but states that short-term exposure to high temperatures should not have an effect. Another recommends a storage temperature of < 27^0C. One importer reported that material was refrigerated before shipment to Australia, and was still cool when it was unpacked from the container; and
- observing shelf life recommendations. A manufacturer reported that under ideal conditions the shelf life of *d*-limonene and related products is 18 months to 2 years. Another estimates the shelf life as 12 months at room temperature if kept away from light.

Peroxide value is one measure of oxidation but does not give a comprehensive picture as it measures only some oxidation products.

Lower-odour grades of limonene are requested by some users, as these are more acceptable to workers. It is believed that odour varies because of varying concentrations of high citrus-odour components in commercial limonene.

Isolation

Isolation as a control measure aims to separate employees, as far as practicable, from the chemical hazard. This can be by distance or enclosure. Isolation when handling limonene was not reported by formulators or users. However, special storage areas are used, related to the flammable rating of limonene under Dangerous Goods legislation.

Engineering controls

The most common engineering control mentioned for the handling of limonene was exhaust ventilation. One laboratory user had high-throughput air conditioning and a histology laboratory used fume hoods and biohazard hoods, reflecting other hazards of their work.

Manufacturers of limonene in Australia reported closed processes of vacuum distillation.

Formulation processes vary in the degree to which the plant is enclosed. Of the methods reported, open processes were most common. Some tanks were closed or semi-closed, and in a few cases, limonene-containing perfume was added to the tank through closed piping.

Safe work practices

Safe work practices are administrative practices that require people to work in safer ways.

Many safe work practices reported for limonene relate to minimising the risks of its flammability. Among these are eliminating any sources of ignition and preventing accumulation of vapours in hollows or sumps.

Oxidation of discarded terpene-soaked rags or other material is a potential fire hazard as spontaneous combustion may occur (Dheri, 1998). A supplier of terpenes offers a proprietary antioxidant for addition to terpene solvents to retard this process. Incidents of this type were not reported during the assessment. Rags are used to apply product or to clean up spills. One formulator of a product containing limonene and tung oil washes any rags to avoid spontaneous combustion of the tung oil, but no precautions against combustion of limonene were reported.

Distillation of limonene occurs during its extraction from orange oil. Precautions against explosive peroxides were not reported, however peroxide levels would be expected to be low in freshly distilled material.

Several safe work practices were reported, as part of general procedures applicable to all products:

- access to formulation area available only to workers on those processes;
- In the absence of local exhaust ventilation, many formulators and users rely on natural ventilation, especially if the work area has no walls or there are large

warehouse doors nearby, or there is cross-flow ventilation. Some users work in the open air, and it was reported that a spray process was done only on "breezy days".

- use of written procedures;
- procedure to ensure workers read MSDS when using a chemical for the first time;
- labelling/placarding of tanks;
- systems that reduce exposure during sampling e.g. sampling tap on tank;
- prompt cleanup of spills; and
- work procedures that cover "worst case" situations.

Personal protective equipment

Where other control measures are not practicable or adequate to control exposure, PPE are used.

For limonene, PPE is primarily used to protect hands and to prevent face and eye splashes. It is usually combined with basic protection such as boots and overalls or wrist to ankle clothing. Aprons were reported to be used by several questionnaire respondents.

Safety glasses and goggles were the most commonly mentioned eye protection, but face shields were also used or available at some workplaces.

PVC and nitrile gloves were used by some formulators and users. Latex was reported to dissolve when used with limonene. Additional information on gloves is provided in Section 14.2.2. Manufacturers recommendations are to be used to choose protective equipment, and suitability may depend on the degree of contact with the chemical. However, one compatibility table for limonene rates nitrile and Viton unsupported gloves materials as most resistant for heavy exposure. Another source endorses use of these materials, as well as polyvinyl alcohol (Forsberg & Mansdorf, 1997).

Respiratory protection is used by a low proportion of formulators and users and several questionnaire respondents emphasised that they did not use it. An organic cartridge respirator is used by graffiti removalists when in confined spaces, and respirators are available if needed at several other companies.

14.2 Hazard communication

14.2.1 Labels

Under the NOHSC *National Model Regulations and Code of Practice for the Control of Workplace Hazardous Substances* (NOHSC, 1994a; NOHSC, 1994b) and the corresponding State and Territory legislation, suppliers and employers of hazardous chemicals used at work are obliged to label hazardous substances in the workplace in accordance with the NOHSC *Code of Practice for the Labelling of Hazardous Substances* (Labelling Code) (NOHSC, 1994c). These requirements also apply to laboratory use, process intermediates, bulk solutions and hazardous substances which are decanted and not consumed immediately. Other labelling requirements, such as those specified under the Australian Dangerous Goods (ADG) Code, also apply.

Under the current NOHSC *List of Designated Hazardous Substances* (NOHSC, 1999a), CAS number 138-86-3 is specifically listed with the names p-mentha-1,8(9)-diene and dipentene. The name limonene or CAS numbers of the *d*- and *l*-isomers are not specifically listed. Guidance to the List states that chemicals with different isomers may be listed generically without mention of each isomer, and that the label text should designate the particular isomer or isomers marketed. In this case, the method of listing may have made the chemical difficult to locate on the List, if *d*- or *l*-limonene was being used rather than dipentene.

A small number of labels for limonene or dipentene were submitted during the assessment. A formal assessment of labels was not carried out. However it was noted that all listed a classification according to the ADG Code, but different UN numbers were used. Some did not clearly list risk and safety phrases applicable to limonene. The risk phrases on some labels varied from those on the NOHSC Designated List.

On some labels a signal word was not stated clearly, and procedures in the case of fire were omitted. These label defects could hinder the handling of an emergency.

The expiry date relevant to the shelf life of the product was not shown on the sample labels, but may be marked on containers in another way.

14.2.2 Material Safety Data Sheets

Material Safety Data Sheets (MSDS) are the primary source of information for workers involved in the handling of chemical substances. Under the NOHSC *National Model Regulations for the Control of Workplace Hazardous Substances* (NOHSC, 1994a) and the corresponding State and Territory legislation, suppliers are obliged to provide an MSDS to their customers for all hazardous substances. MSDS should be prepared in accordance with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 1994d). A sample MSDS for *d*-limonene is included in Appendix 4, as this is the isomer most in use in Australia.

MSDS were provided by 14 suppliers of limonene or dipentene in Australia and one supplier of a product with a high limonene content. All were dated and were issued between 1997-2001. Although a formal assessment was not carried out, the following points were noted regarding the different sections of the MSDS:

Statement of hazardous nature

- Three MSDS omitted this statement. One stated incorrectly that the chemical is not considered hazardous;

Identification

- Products were described as limonene (*d*- or *dl*-) and dipentene, but also often as "Terpene hydrocarbons NOS (*d*-limonene)", reflecting the shipping name commonly assigned to *d*-limonene. Commercial grades of dipentene containing < 50% limonene were identified as "terpene hydrocarbons" or "pine oil 65%". Pure (> 95%) grades of *d*-limonene were also identified as "citrus terpenes", "orange terpenes" or "orange oil terpenes";

- Almost all MSDS included the correct Hazchem code 3[Y] and Packing Group (III). The UN number assigned to dipentene (2052) was also used by some suppliers for *d*-limonene. Other suppliers of *d*-limonene used UN 2319, which is the code for Terpene Hydrocarbons NOS. The MSDS for a grade of pine oil containing limonene was coded as UN 1272; and
- In general the MSDS contained substantial information on physical description and properties. However, the important parameters boiling point, vapour pressure, and flashpoint were missing from some MSDS, and 5 did not have data for flammability limits.

Health hazard information

- Most MSDS distinguished between acute and chronic effects, and routes of exposure;
- Skin irritation is a listed effect under the NOHSC *List of Designated Hazardous Substances* (NOHSC, 1999a). All MSDS acknowledged an effect on skin, although a few described the effects as redness, drying or defatting rather than irritation. Dermatitis was mentioned without distinguishing between irritant contact dermatitis and allergic contact dermatitis;
- The skin sensitisation effect of limonene is not yet listed in the NOHSC *List of Designated Hazardous Substances* (NOHSC, 1999a). However, this effect was mentioned on 6 MSDS;
- Eye irritation was mentioned on almost all MSDS; and
- Most first aid instructions recommended that vomiting should not be induced, but did not explain that this is primarily to avoid aspiration.

Precautions for use

- Most MSDS advised that there was no NOHSC exposure standard but a few did not cover the topic at all;
- Ventilation was the main engineering control mentioned, and advice varied from exhaust ventilation to "no special requirements". A few MSDS noted that as limonene vapour is heavier than air, it will collect in low areas and that extraction should deal with this vapour accumulation;
- Glove material most commonly recommended in MSDS was PVC, rubber, neoprene or butyl. Other materials have been reported as having high resistance to limonene (Section 14.1). Eye protection was recommended in all MSDS. For exposure to a high vapour level or in confined space, self contained breathing apparatus (SCBA) was generally recommended;
- Several MSDS wrongly identified limonene as a combustible liquid instead of a flammable liquid. Precautions to avoid fire were not included in this section in 2 MSDS, and in general were covered only briefly; and
- Only one MSDS covered the risk of spontaneous combustion of rags soaked in limonene and the risk of distilling material containing peroxides.

Safe handling information

- Information on storage should cover both the flammability and stability aspects of the chemical, but this was done in only a small number of MSDS;

- Transport classification information was stated on most MSDS, and some also included information on segregation of different classes of dangerous goods;
- Information on spills and disposal often did not distinguish procedures for small and large spills; and
- The primary fire extinguishing materials recommended in MSDS were dry chemical, CO_2 and foam. Varied advice was given on use of water in firefighting. In general MSDS did not comprehensively cover all the factors relevant to limonene in a fire – flammability, explosion hazard, immiscibility with water, high vapour density and ability to accumulate static charge.

Contact point

- A majority of MSDS included contact information in Australia, and indicated whether this was available 24 hours a day or only during business hours.

14.2.3 Education and training

No specific training for the use of limonene was reported, but several companies reported general training in use of chemicals and reading MSDS. One advised that all workers were taught about dangerous goods and the use of fire extinguishers.

Guidelines for the induction and training of workers exposed to hazardous substances are provided in the NOHSC *Model Regulations and Code of Practice for the* Control *of Workplace Substances* (NOHSC, 1994a; NOHSC, 1994b). Under the regulations employers are obliged to provide training and education to workers handling hazardous substances.

14.3 Occupational monitoring and regulatory controls

14.3.1 Monitoring

Under the NOHSC Model Regulations (NOHSC, 1994c), employers are required to carry out an assessment of the workplace for all hazardous substances, the methodology of which is provided in the NOHSC *Guidance Note for the Assessment of Health Risks Arising from the Use of Hazardous Substances in the Workplace* (NOHSC, 1994f). When the assessment indicates that the risk of exposure via inhalation is significant, atmospheric monitoring should be conducted to measure limonene levels in the workplace as a precursor to the implementation of suitable control measures to reduce exposure. Subsequent monitoring will be required to ensure that such measures are effective.

No air or biological monitoring was reported for limonene, although a few companies noted that they had monitored for other substances.

14.3.2 Hazard classification

Workplace substances are classified as hazardous to health if they meet the NOHSC *Approved Criteria for Classifying Hazardous Substances* (the Approved Criteria) (NOHSC, 1999).

The health hazard assessment of limonene indicates that:

- *d*-Limonene has low acute toxicity;

- Limonene is a skin irritant in rodents. There are limited data on the potential of limonene to cause eye or respiratory irritation;
- The autoxidation products of limonene are skin sensitisers. Limited data are available on the potential for limonene to cause respiratory sensitisation;
- Following repeated exposure to limonene in male rats, the target organ is the liver with increased liver weights reported. However this effect is thought to be due to enzyme induction;
- Renal tumours found in male rats are sex and species specific. The mechanism is considered to be related to the protein $\alpha 2\mu$-globulin and is not relevant to humans;
- Limonene is not mutagenic; and
- Limonene has no reprotoxicity or teratogenic potential.

Limonene is currently included in the NOHSC 1999 *List of Designated Hazardous Substances* with the classification in Table 14.1. The material is identified as CAS number 138-86-3 and the listed names are p-mentha-1,8(9)-diene and dipentene.

The current European Union classification for limonene is also tabulated in Table 14.1. The classification covers *d*-, *l*- and *dl*- limonene (dipentene) as well as trans-isolimonene (not included in this assessment). The hazard assessment for limonene supports this classification, which will be taken up by NOHSC at the next revision of the *List of Designated Hazardous Substances.*

Liquid substances and preparations presenting an aspiration hazard in humans because of their low viscosity are to be classified as R65 Harmful: May cause lung damage if swallowed. This applies to:

- substances containing aliphatic, alicyclic and aromatic hydrocarbons in a total concentration equal to or greater than 10% and having viscosity and surface tension values as specified (NOHSC, 1999); or
- substances and preparations, based on practical experience in humans.

Limonene and its preparations may meet these criteria. Depending on test results of various grades of limonene and limonene products, classification must be carried out by suppliers based on the characteristics of the material supplied.

Table 14.1 - Current Australian and European Union hazard classification for limonene

	Australian hazard classification (NOHSC, 1999a)	European Union classification (European Commission, 1998)
Classification and Risk phrases#	**Irritant X_i R38** **R10* -** Flammable **R38** – Irritating to skin	**Irritant X_i R38 R43** **R10* -** Flammable **R38** – Irritating to skin **R43** – May cause sensitisation by skin contact **Environmental N*** **R50*** – Very toxic to aquatic organisms **R53*** – May cause long term adverse effects in the aquatic environment
Safety phrases#	**S(2)##** – Keep out of reach of children **S28 –** After contact with skin, wash immediately with plenty of soap-suds.	**S(2)## -** Keep out of reach of children **S24 -** Avoid contact with skin **S37 -** Wear suitable gloves **S60* –** This material and/or its container must be disposed of as hazardous waste **S61* -** Avoid release to the environment. Refer to special instructions/ safety data sheet
Concentration cut-off levels and classification for mixtures**	**At concentrations ≥ 25% Irritant X_i** **R38** – Irritating to skin	**At concentrations ≥ 20% Irritant X_i** **R38** – Irritating to skin **R43 –** May cause sensitisation by skin contact **At concentrations ≥ 1% & < 20% Irritant X_i** **R43 –** May cause sensitisation by skin contact

*Physico-chemical and environmental classification and risk phrases are not required under Australian workplace regulations and are provided for information only.
** Occupational risk phrases only are listed for mixtures.
# Where the criteria for aspiration hazard are fulfilled, the risk phrase "R65 – Harmful: May cause lung damage if swallowed" and the corresponding safety phrase "S62 –If swallowed, do not induce vomiting; seek medical advice immediately and show this container or label" should also be used.
The safety phrase S2 can be omitted from the label when the substance or preparation is sold for industrial use only.

Note C: Some organic substances may be marketed either in a specific isomeric form or as a mixture of several isomers.
[Under 'Name:' or 'Synonyms' in the Full Text field], a general designation of the following type is sometimes used:"xylenol"
In this case the manufacturer or any other person who markets such a substance should state on the label whether the substance is a specific isomer (a) or a mixture of isomers (b).
Example: a) 2,4-dimethylphenol b)xylenol (mixture of isomers)

14.3.3 Exposure standard

No national occupational standard has been assigned by NOHSC to limonene. Current overseas occupational exposure standards for limonene are summarised in Table 14.2.

Supporting documentation for the exposure standard adopted by American Industrial Hygiene Association (AIHA) states that the 30 ppm 8-hr TWA was set to protect against liver effects seen in male mice and reduced survival in female rats in a 2-yr NTP study (AIHA, 1993).

Table 14.2 - National occupational exposure standards for limonene

Country	8 h TWA	STEL	Year Adopted	Reference
Norway (OEL)	25 ppm (140 mg/m^3)	-	1999	(RTECS, 2001)
Sweden	25 ppm (150 mg/m^3)	50 ppm (300 mg/m^3)	1990	(Karlberg & Lindell, 1993)
Finland	25 ppm (140 mg/m^3)	50 ppm (280 mg/m^3)	not known	(Finnish Institute of Occupational Health, 2001)
AIHA	30 ppm (165.6 mg/m^3)	-	1993	(AIHA, 1993)

OEL = Occupational exposure limit

STEL = short-term (15-min) exposure limit

TWA = time-weighted average

In Germany no MAK (maximum workplace concentration) has been set for limonene as it was considered that insufficient information was available. However all isomers have been listed as skin sensitisers (Deutsche Forschungsgemeinschaft, 2000).

14.3.4 Health surveillance

In accordance with NOHSC *National Model Regulations for the Control of Workplace Hazardous Substances* (NOHSC, 1994b), employers have to provide health surveillance in those workplaces where the workplace assessment indicates that exposure to a hazardous substance may lead to an identifiable substance-related disease or adverse health effect. Limonene is not listed in Schedule 3 (list of substances requiring health surveillance) and as such there are no formal requirements for health surveillance programs for exposed workers.

No occupational health surveillance was reported for limonene in Australia.

14.3.5 Australian Code for the Transport of Dangerous Goods by Road and Rail

Under the ADG Code (FORS, 1998), limonene (dipentene) (UN Number 2052) is classified in Class 3, Packing Group III. Class 3 contains principally flammable liquids. Limonene is assigned Packing Group III (because of its high flash point), indicating that it is of lower hazard than most other flammable liquids. Packing Groups are assigned according to the degree of risk the goods present in transport. Packing Group III indicates minor danger.

The *d*- and *l*- isomers are not mentioned specifically under UN Number 2052. As the physico-chemical properties and flammability hazard of the isomers would be similar to the racemic mixture, this number could also be used for *d*- and *l*-limonene. An alternative classification used by several companies is the generic category Terpene Hydrocarbons N.O.S., which is identified as UN Number 2319, and has the same class, Packing Group and Hazchem Code as limonene/dipentene.

The ADG Code sets out various requirements relating to the packaging, labelling and transport of limonene by road or rail.

Bulk loads must be placarded with Class label 3 ('flammable liquid') and an emergency information panel containing additional information such as the Proper Shipping Name of the dangerous goods ('dipentene'), its UN Number, Hazchem Code and the name and telephone number for specialised advice. The Hazchem Code for bulk loads of limonene is 3[Y]. The code reflects the initial emergency response recommended in case of fire, leakage or spillage. The number '3' indicates that foam should be used for firefighting. The letter '[Y]' means that there is a risk of violent reaction or explosion; that emergency personnel should wear a breathing apparatus a fire situation; and that any spillage should be contained so as to prevent the chemical from entering drains or water courses.

Additional information used in transport includes Emergency Procedure Guides (EPG) for individual dangerous goods, or the generic Standards Australia "Dangerous Goods – Initial Emergency Response Guide" (SAA/SNZ, 1997), also known as Handbook 76 or HB76. In this document, transporters of UN 2052 and UN 2319 are directed to guides 14 (Liquids – Highly Flammable) and 15 (Liquids – Flammable) respectively.

Under the ADG Code, controls on the transport of smaller quantities of limonene and limonene products within Australia are based primarily on package and labelling requirements.

14.3.6 Storage and handling

The NOHSC *National Standard for the Storage and Handling of Workplace Dangerous Goods* (NOHSC, 2001) and *National Code of Practice for the Storage and Handling of Workplace Dangerous Goods* (NOHSC, 2001a) form a performance-based foundation for a national framework of control, incorporating the principles of hazard identification, risk assessment and risk control. Implementation of the standard will be via States and Territories.

Existing State and Territory arrangements include requirements in NSW for licensing in that State of premises holding dangerous goods above certain threshold quantities. Information in the Stored Chemicals Information Database (SCID) is used to inform emergency services staff such as fire fighters.

14.4 Public health controls

14.4.1 Labels

A small number of labels for consumer products containing limonene were submitted for review. Most of these products contained limonene as a fragrance compound at low concentrations (generally 1% or less). At these concentrations, limonene would be expected to have a limited contribution to the toxic hazards of the products. One household cleaning product contained 5% *d*-limonene, and two hand cleaners contained 9.75% and 15% of *d*-limonene. There were no precautionary or warning statements concerning eye irritation potential on the labels for these products. There were no statements concerning skin irritation potential for the household cleaner, although the product label "recommended gloves for sensitive skin". Skin irritancy would not be expected from the hand cleaners, as such an attribute would lead to discontinuation of use by consumers and would be commercially undesirable.

14.4.2 Public health regulatory controls

At present, there are no public health regulatory controls for limonene. Although limonene is a liquid hydrocarbon ($C_{10}H_{16}$), it is not included in the SUSDP entry for liquid hydrocarbons, which specifically includes kerosene, diesel (distillate), mineral turpentine, white petroleum spirit, toluene, xylene, and light mineral and paraffin oils (but excludes their derivatives). Lemon, lime, and bitter orange oils, containing 42 to > 90% limonene (NDPSC, 1998) are Schedule 5 entries in the SUSDP as they may contain photosensitising components such as furanocoumarins.

14.5 Environmental regulatory controls

When imported and used as outlined in this report, limonene is not expected to cause significant adverse environmental impact. Environment Australia is not aware of any environmental regulatory controls for the use of limonene in Australia. As such, Environment Australia is not recommending any specific regulatory controls for the use of this chemical in Australia.

In the EU limonene has been assigned the following risk phrases:

R50 - Very toxic to aquatic organisms and

R53 - May cause long-term adverse effects in the aquatic environment.

The assignment of these phrases is based on the substance having acute toxicity: to fish (96 h LC50 $\leq$ 1 mg/L), daphnia (48 h EC50 $\leq$ 1 mg/L) or algae (72 h IC50 $\leq$ 1 mg/L) and the substance not being readily degradable or the log Pow (log octanol/water partition coefficient) $\geq$ 3.0 (unless the experimentally determined bioconcentration factor(BCF) $\leq$ 100).

In accordance with the Harmonised Integrated Hazard Classification System for Chemical Substances and Mixtures limonene would be classified in the Acute I Class (OECD, 2001). This classification is based on the 96 h LC50 (fish) $\leq$ 1 mg/L and/or 48 h EC50 (for crustacea) ≤ 1 mg/L and/or 72 or 96 h EC50 (for algae or other aquatic plants) ≤ 1 mg/L. Under this classification system limonene would also be classified in the Chronic I class which has the same toxicity specifications as the Acute I class with the additional stipulation that the substance is not rapidly degradable and/or the log Kow ≥ 4 (unless the experimentally determined BCF < 500).

14.5.1 Disposal and waste treatment

The disposal information contained in the submitted MSDSs varied substantially. The following statement is representative and which Environment Australia believes to be satisfactory:

Spills	Use protective gloves to avoid skin contact. Ventilate area thoroughly and wear a respirator if necessary to minimise inhalation. Eliminate any source of ignition and do not smoke. Small spills can be wiped up. Rags or other combustible material wet or soaked in limonene may autoxidise, generating heat and igniting spontaneously. Used oily rags should be collected regularly and either soaked in water or stored in closed metal containers. Large spills should be absorbed by dirt, sand, or other suitable absorbents for disposal.
Disposal	Do not hose spills down drains, sewers, or waterways. *d*-Limonene may be toxic to aquatic organisms. Move leaking containers to well ventilated area. Contact your local waste disposal authority for advice, or pass to a licensed waste disposal company for disposal.

14.6 Emergency procedures

For any hazardous chemical an emergency response plan is an essential component of occupational health. In the event of a substantial release or a fire, a written procedure is necessary for workers and emergency services. For limonene and dipentene the most relevant emergency conditions are large spills and fire. Some relevant information was supplied on MSDS. No emergency plans were supplied for assessment.

Emergency plans should cover first aid, arrangements for medical care, containment and clean-up of spills and actions in case of fire.

15. Discussion and Conclusions

15.1 Manufacture, importation and uses

Approximately 100 to 130 tonnes of *d*-limonene is manufactured per year in Australia from orange oils by extraction through distillation. There is no report of dipentene and *l*-limonene manufacture in Australia.

Approximately 1500 tonnes of *d*-limonene and dipentene were imported each year from a number of countries. Products containing about 60 tonnes of limonenes (*d*-, *l*-, *dl*-) per year are also introduced into Australia and the concentrations of limonene in imported products vary widely, from very low to 90%. Commercially supplied dipentene varies greatly in purity, and impurities may vary from those in *d*-limonene.

d-Limonene and dipentene are mainly used in formulation of a range of fragrance or flavour blends that are used to further formulate end products for industry and consumer use or as food additives. They are also used in the direct formulation of a large range of industrial and consumer products, mainly cleaning and degreasing products, as replacement for toxic chlorinated solvent or as solvent carriers. Other products include fire extinguishant, paints, car detailing products, timber finishes, paper softener, masking agent and wire rope lubricant. Some pure *d*-limonene and dipentene are also used directly in cleaning and degreasing, wax removing, microbiological and gemstone testing and as a general reagent in laboratories. Availability of pure limonene to the general public was not reported.

Limonene products are either used directly or further diluted or mixed with other components by end users. Industrial products containing limonene are mainly hand cleaners, industrial cleaning and degreasing products, and removers and strippers. The final concentration of limonene in industrial products formulated in Australia varies widely and ranges from < 1% to 95%. Limonene is used in consumer products mainly as flavouring and/or fragrance agents in food, pharmaceuticals and household and cosmetic products. Limonene is also present in essential oils that are widely used in Australia. The concentration of limonene in the final consumer products also varies largely and usually is low, but can be as high as 70%.

It should be noted that the information on uses of limonene provided by industry and obtained by the NICNAS phone survey is limited as there may also be other uses of limonene not identified by NICNAS. The profile of users contacted during the NICNAS phone survey might not be representative as only a small proportion of formulators and users of limonene were contacted.

15.2 Environment

Limonene is a volatile chemical with slight water solubility. Significant releases are expected to the aquatic compartment as a result of the use pattern in cleaners. However, as a result of dilution, degradation and evaporation these releases are not expected to result in adverse effects on aquatic organisms. No studies were identified on chronic effects, and therefore risks associated with chronic exposures of aquatic organisms to limonene in "polluted" waters cannot be determined.

Due to the high volatility of limonene the atmosphere is expected to be the major environmental sink for this chemical, where it is expected to rapidly undergo gas-phase reactions with photochemically produced hydroxyl radicals, ozone, and nitrate radicals. The oxidation of limonene may contribute to aerosol and photochemical smog formation. Ozonolysis of limonene may also lead to the formation of hydrogen peroxide and organic peroxides, which have various toxic effects on plant cells and may be part of the damage to forests observed in the last decades. Emissions of biogenic hydrocarbons such as limonene and other terpenes to the atmosphere may either decrease ozone concentrations when oxides of nitrogen concentrations are low or, if emissions take place in polluted air (i.e. containing high oxides of nitrogen levels), lead to an increase in ozone concentrations (IPCS, 1998). Limonene has not been identified as an air toxic in Australia and is not on the list of substances reported to the National Pollutant Inventory.

15.3 Health hazards

d-Limonene is readily absorbed by inhalation and ingestion. Dermal absorption is reported to be lower than the inhalation route. Limonene has been used to enhance the dermal penetration of drugs, and its effect is believed to be due to lipid disruption in the stratum corneum. *d*-Limonene is rapidly distributed to different tissues in the body and is readily metabolised, with several possible pathways of metabolism. Elimination occurs primarily through the urine.

Limonene is a skin irritant in both experimental animals and humans. It has the potential to cause eye irritation and respiratory irritation, but data on these effects are limited. Changing concentrations of limonene in indoor air have been shown to cause changes in the responsiveness of breathing airways. Acute toxicity of limonene is low.

Studies in guinea pigs revealed that autoxidized *d*-limonene induced contact allergy. As *d*- and *l*- are enantiomers, this could be true for *l*-limonene and dipentene (the mixture). Limonene is classified as a skin sensitiser and it is believed the effect is due to oxidation products rather than limonene itself. Limited data are available on the potential to cause respiratory sensitisation.

Renal tumours are induced by limonene in male rats and these are thought to be sex and species specific and are not considered relevant to humans. Other than the kidneys, the critical organ in animals is the liver, with effects having been observed in mice, rats and dogs. Exposure affects the amount and activity of liver enzymes, liver weight, cholesterol levels and bile flow. However, no toxic effects on the liver have been reported. The liver effects in animals are thought to be due to physiological adaptation. From the data available, it is not possible to identify a NOAEL for these effects.

In general, limonene could be considered to be a chemical with fairly low toxicity, with exception of its irritative and sensitising properties.

15.4 Occupational health and safety

Limonene is a flammable liquid with a flash point of 48^0C. Its flammability limits in air are 0.7% to 6.1% by volume. Explosive vapour/air mixtures may be formed at temperatures above 48°C. It may decompose to produce toxic fumes of carbon monoxide. Rags or other combustible material that have been wet or soaked with limonene may spontaneously combust. Distillation to dryness may also lead to concentration of peroxides and the risk of explosion. However, information provided by industry indicated that the material is manufactured in an enclosed system and is transported and stored according to the regulations for Class 3 flammable goods. The fire/explosion risk, therefore, is significantly reduced. The risk of fire/explosion during repacking, formulation and end use can be minimised if exposure of limonene to heat, static spark, open flame or other ignition sources could be avoided.

Under the ADG Code, *dl*-limonene (dipentene) has the specific UN number 2052. The *d*- and *l*- isomers are not mentioned specifically under this number. As the physico-chemical properties and flammability of these isomers would be similar to those of the racemic mixture, this UN number could also be used for *d*- and *l*-limonene.

Additional information used in transport includes the generic Initial Emergency Response Guides (IERG) produced by Standards Australia / Standards NZ. It is considered that UN 2052 would be more suitably linked to generic Guide 15, rather than Guide 14 as at present, based on flammability.

Autoxidation of limonene occurs readily in the presence of light and air forming a variety of oxygenated monocyclic terpenes. The sensitising properties of limonene are acknowledged to be due to its oxidation products. Risk of skin sensitisation is high in situations where oxidation products of limonene are likely to be formed during use of limonene. However, autoxidation of limonene can be controlled by addition of antioxidant, protection against air, light and elevated temperature, and monitoring shelf life. Inclusion of oxidised limonene in patch testing for allergy would be a useful diagnostic tool.

Information on the total number of workers handling limonene during manufacture, importation, repacking, formulation and use in Australia is not available, but it is expected to be large, especially for end use. Occupational exposure during manufacture, importation, transportation and storage of limonene is limited, except in cases of accidental spills or leaks of the chemicals. Therefore the risk of developing any adverse effects is low. During repacking and formulation, operators are likely to be exposed by skin and eye contact during manual operations and cleaning of equipment. There is also a potential for inhalation exposure, especially when working in a confined space and in places with limited ventilation, heated blending process, high speed mechanical stirring and at worksites with an open mixing process and no exhaust ventilation. The potential for worker exposure during use of limonene or limonene products is high based on the widespread end uses, modes of use and lack of control measures at some worksites.

A number of areas of concern have been identified in this assessment. There is a risk of skin, eyes and respiratory irritation from working in confined spaces and in places with limited ventilation e.g. cleaning of storage tanks, and during end use of limonene and/or products containing high concentrations of limonene. Repeated exposure to these conditions would increase the risk of health effects. Hand cleaners are a product category where dermal contact with limonene is deliberate rather than incidental, suggesting that caution in the use of these products is warranted, and that control of oxidation is critical. "Wash-off" hand cleaners are preferable to "wipe-off" products.

Inadvertent exposures to limonene can be controlled by use of an automated or enclosed system, decontamination of equipment where appropriate, use of local exhaust ventilation and PPE. Where limonene mists/aerosols may be generated, potential inhalation exposure can be also controlled by using anti-mist additives in the formulations. Respiratory protection suitable for normal working conditions may not be suitable for confined spaces. The minimum requirement for working in a confined space is set out in the Australian Standard AS 2865 *Safe Working in a Confined Space* (Standards Australia, 1995).

Based on the information available during this assessment, and the results of the risk characterisation, establishment of an occupational exposure standard is not warranted.

15.5 Public health

Limonene is naturally present in the food we eat and in the air we breathe. It has been shown to be present at higher concentrations in indoor air than outdoor air, possibly due to the use of air-fresheners and cleaning products with fragrant residues. It occurs in a diverse range of consumer products due to its fragrant, solvent, and insecticide properties, or combinations of these properties for use in fragrances, cosmetics and toiletries, cleaning products, processed foods, and household insecticides. As a solvent for cleaning purposes, limonene is of a similar performance but generally of low toxicity when compared with other traditional solvents e.g.. acetone, xylene, etc.

Although there are a large number of products containing limonene in the public domain, most contain limonene at low concentrations ($\leq$ 1%). As a minor ingredient, limonene would consequently have a minimal contribution to the toxic hazards of these products. In other consumer products such as certain solvent-type cleaning agents, limonene can be present at concentrations of up to 60%. At these concentrations, limonene may present an irritation hazard to the eyes and/or skin, with the potential for oxidised forms of limonene to be skin sensitisers in sensitive individuals. Consequently, this report will be referred for consideration of scheduling by the NDPSC. Limonene is currently not included in the *Standard for the Uniform Scheduling of Drugs and Poisons* (NDPSC, 2001).

15.6 Concluding remarks

This assessment report drew largely on the internationally agreed Concise International Chemical Assessment Document (CICAD) for the hazard assessment. The major hazards are irritation and sensitisation. The assessment focussed on use and exposure in Australia and enabled a number of recommendations to be made for the Australian situation, including hazard classification for labels and MSDS.

15.7 Data gaps

A number of gaps have been identified from the available data for limonene. They are:

- workplace atmospheric monitoring;

- health effects of limonene in humans following repeated exposures;
- data on the permeability of limonene through the skin;
- information on level of limonene in products to pose an irritation or sensitisation hazard;
- data on autoxidation of limonene in mixtures.

16. Recommendations

Recommendation 1: Hazard classification

Limonene is classified as a hazardous substance by NOHSC, and listed under CAS number 138-86-3 and the names p-mentha-1,8(9)-diene and dipentene. The current EU classification, adopted in Commission Directive 98/73/EC, also classifies the chemical as a skin sensitiser, and this classification is due to be taken up in the next update of the NOHSC *List of Designated Hazardous Substances.* The classification also applies to CAS numbers 5989-27-5 and 5989-54-8 and to the names limonene, *d*-limonene and *l*-limonene, and has labelling note C. This assessment has not identified data contrary to the EU classification. Therefore it is recommended that the EU classification be adopted, including:

- In addition to the current classification of X_i, R 38 (Irritating to skin) limonene also be classified as a skin sensitiser with the risk phrase

  R43 May cause sensitisation by skin contact

  This amendment should be taken up in the NOHSC List of Designated Hazardous Substances as soon as possible. Further information on classification and labelling is contained in Table 14.1. Limonene products containing ≥ 20% limonene are classified with risk phrases R38 and R43. Limonene products containing ≥ 1% & < 20% limonene are classified with risk phrase R43.

- Recommended occupational safety phrases include:
  S2 – Keep out of reach of children
  S24 – Avoid contact with skin
  S37 – Wear suitable gloves
  S62 (only if classified as R65) – If swallowed, do not induce vomiting; seek medical advice immediately and show this container or label

- Where limonene/dipentene or products containing it meets the criteria for aspiration hazard, the following risk phrase should be used:

  R65 Harmful: May cause lung damage if swallowed

- Suppliers should ensure that commercially obtained natural extracts containing substantial amounts of limonene are classified to reflect this content. Examples of materials where the limonene content may not currently be acknowledged are "Terpenes and Terpenoids, orange-oil, CAS No 68647-72-3" and "Hydrocarbons, terpene processing by-product, CAS No 68956-56-9" (see Section 4).

Recommendation 2: Hazard classification of chemicals that may form hazardous products on storage

It is recommended that NOHSC consider reviewing the general issue of classification of chemicals which may form hazardous products on storage, and if appropriate develop guidelines that will ensure consistent classification.

Recommendation 3: Prevention of formation of oxidation products

It is recommended that industry take steps to prevent as far as possible the formation of oxidation products in limonene/dipentene, throughout its life cycle. A combination of the following measures could be used towards this aim:

- storage to minimise contact with air, light and heat;
- correct choice of packaging;
- addition of antioxidants;
- precautions in handling;
- monitoring of peroxide levels; and
- use of expiry dates.

A model from one industry sector is the IFRA Guideline, which specifies that:

"*d*-, *l*-, and *dl*-limonene and natural products containing it should only be used when the level of peroxides is kept to the lowest practical level, for instance by adding antioxidants at the time of production. Such products should have a peroxide value of less than 20 millimoles peroxides per litre, determined according to the FMA method." (see Appendix 5)

Recommendation 4: Workplace controls

It is recommended that workplaces manufacturing, handling or using limonene/dipentene, take note of the possibility of spontaneous flammability of used rags or other combustible material in contact with limonene, and of the explosive potential of limonene containing peroxides, when concentrated or distilled to dryness. Appropriate education and measures to control these hazards is recommended.

Engineering controls and safe work practices should be implemented to avoid dermal and inhalational exposure to limonene that may contain oxidation products, and to concentrations of limonene that may be irritating. NICNAS will prepare a Safety Information Sheet for limonene, for display in workplaces and highlighting appropriate control measures.

Recommendation 5: Hazard Communication

Suppliers of limonene/dipentene and products must comply with the NOHSC requirements for hazardous substances.

It is recommended that

- Labels and MSDS be amended to incorporate the correct classification.
- all manufacturers, suppliers and employers review their hazard communication, including MSDS compliance, paying particular attention to the following points:

- correct identification of health hazards;
- inclusion of all information relevant to the flammability hazards;
- guidance to avoid spontaneous combustion of used rags or other combustible material;
- guidance on the potential for explosion if peroxides are concentrated;
- first aid advice, including the advice that vomiting should not be induced;
- glove material suitable for the recommended uses; and
- information on handling of spillages.

Emphasis should be given to relevant hazards, depending on the use of limonene in workplaces. For example, management of used rags should be a high priority in wipe-cleaning applications. Peroxides are potentially explosive when oxidised material is re-distilled, but this procedure is unlikely to be carried out in most applications of limonene.

Although there are not sufficient data to classify limonene as an eye irritant under the NOHSC *Approved Criteria for Classifying Hazardous Substances*, it is recommended that MSDS include warnings on eye irritation, and guidance on eye protection, because eye irritation in rabbits has been reported.

Recommendation 6: ADG code

It is recommended that the Department of Transport and Regional Services (DOTARS) consider a submission to the UN to include *d*-limonene under the same UN code as *dl*-limonene, for consistency.

Recommendation 7: Initial Emergency Response Guide

It is recommended that Standards Australia consider whether UN2052 should be covered by the Initial Emergency Response Guide 15, rather than Guide 14 as currently listed, on the basis of limonene's lower (Packing Group III) flammability.

Recommendation 8: Patch testing of hand eczema patients

It is recommended that medical practitioners carrying out patch testing for allergy diagnosis consider inclusion of oxidised limonene in the test series.

Recommendation 9: Public health regulatory controls

It is recommended that the final PEC report be forwarded to the National Drugs and Poisons Schedule Committee for their consideration, due to concerns about irritation or sensitisation in products containing higher concentrations of limonene.

The adoption of IFRA guidelines would remove the sensitisation hazard in those product covered by these guidelines.

17. Secondary Notification

Under Section 65 of the IC(NA) Act, secondary notification may be required where a manufacturer and/or introducer of the existing chemical(s) becomes aware of any circumstances that may warrant a reassessment of its/their hazards and/or risks. Such circumstances include:

a) the function or use of limonene has changed or is likely to change significantly;

b) the amount of limonene manufactured and/or introduced into Australia has increased, or is likely to increase significantly;

c) additional information has become available to manufacturers/introducers as to the adverse health, safety or environmental effects of limonene, such as the revision of hazard classification(s) undertaken by the EU.

The Director must be notified within 28 days of the supplier (manufacturer/importer) becoming aware of any of the above, or other circumstances prescribed under section 65 of the IC(NA) Act.

Appendix 1

LIMONENE SURVEY FORM
(CAS No. 5989-27-5, 5989-54-8, 138-86-3)

NOTES for caller:

Following points need to be addressed before start of the survey:

1. Identify who you are; *(can say what NICNAS does and refer them to website for further information or offer to tell them more)*
2. What you are after; *(information to aid the assessment that can't be provided by importer)*
3. Ask who is the best person to talk to in the company (ask for Regulatory Affair Officer first); *(when speaking to the right person, check that they have a few minutes to answer the questions, and if not a suitable time at present arrange to ring them back at an agreed time)*
4. How NICNAS gets the company (mention the supplier's name);
5. The role of the company
   - reseller? - Form 1
   - repacker? - Form 2
   - formulator? - Form 3
   - end user? - Form 4

6. Assurance that identity of company will not be disclosed and that data will be collated with that of other companies.

Company name:__________________________________

Contact person's name:______________________________

Phone No.____________________

Fax No.______________________

The role of the company____________________________

Notes:

7. At end of interview thank them for their help. Ask if they would like to be sent a safety info sheet at the end of the assessment. If they would like this, make sure we have sufficient contact details.

Limonene Survey Form 1

This questionnaire applies to companies that <u>only resell</u> LIMONENE or product(s) containing LIMONENE.

---

1. Please provide the following details for the products your company uses:

Product Name	% limonene	Typical end uses (if known)

2. Please provide customer list as soon as possible.

<u>Please ensure the company that the information will be kept confidential!!</u>

Thank You for Responding to the Survey!!

If you have any queries about the survey or any of the questions, please contact:

Dr Jun Zhang

Phone 02 9577 9577
Fax: 02 9577 9465
Email: jun.zhang@nicnas.gov.au

Limonene Survey Form 2

This questionnaire applies to companies that repack LIMONENE or product(s) containing LIMONENE.

---

1. Please provide the following details for products your company repacks:

Product Name	% limonene	Type of product	Package size	Typical end uses

2. Please describe general packing processes for your products.

Following questions need to be answered for this point:

1. How Limonene or products containing Limonene is transferred to packing area?
2. Open or closed packing process (automated or manual? How to put cap on container?)
3. Is it a continuous process?

____

____

____

3. How is the equipment used during repacking LIMONENE products cleaned AND how you dispose any cleaning residues?

____

____

____

____

4. **Please describe numbers and activities of workers repacking LIMONENE products.**

Number	Description of Work	Hrs/day	Days/yr

5. **Please describe the engineering controls that are in place to reduce exposure of workers to LIMONENE eg. exhaust ventilation, general dilute ventilation.**

Process/Activity	Engineering Controls	Year installed

6. **Please give details of the personal protective equipment used by workers. eg type of gloves, goggles, respirators, protective clothing.**

Process/Activity	Personal Protective Equipment	Type

7. **Are any other precautions taken to reduce exposure of workers to LIMONENE?**

Limited access to area of use, Written procedures for safe use,

Special labeling or placarding. Other________________________

8. **Has atmospheric monitoring been conducted to determine levels of LIMONENE in the workplace?** Yes No

If yes, please provide details of monitoring eg. testing equipment, methods, duration, and results.

____

____

____

9. **Have there been any incidents involving spillage of LIMONENE or LIMONENE products at your workplace AND how they are cleaned?**

Yes No

If yes, please give details:

____

____

____

10. **Are you aware of any adverse health effects (eg. skin, eye and respiratory irritation) experienced at your workplace due to exposure and/or spillage of LIMONENE or LIMONENE products?**

Yes No

If yes, please give details:

____

____

____

Environmental Effects

11. Is there any LIMONENE waste generate during repacking?

Yes No

If yes, what type?

____

12. What methods do you use to dispose LIMONENE waste?

Blending with other products and re-use

Evaporation to atmosphere

Licensed discharges

Incineration eg. boiler fuel (Cont.)

Send to recycler

Waste collection

Other (please specify)______________________________

13. Please indicate how you handle LIMONENE or LIMONENE products empty containers.

Rinse and/or re-use Return to supplier

Sell to drum recycler Send to landfill

Other (please specify) ______________________________

Thank You for Responding to the Survey!!

If you have any queries about the survey or any of the questions, please contact:

Dr Jun Zhang

Phone 02 9577 9577
Fax: 02 9577 9465
Email: jun.zhang@nicnas.gov.au

1

Limonene Survey Form 3

This questionnaire applies to companies that <u>manufacture/formulate</u> product(s) containing LIMONENE. Simple dilution is counted as formulation.

1. Please provide the following details for products you formulate:

Product Name	% limonene	Type of product	Package size	Typical end uses

2. Please describe general manufacturing processes for your products.

Following questions need to be answered for this point:

1. How limonene is transferred to manufacture area?
2. Open or closed manufacturing process?

   Hints:
   - Open (eg. open tanks, LIMONENE added to tanks manually)
   - Partially closed (eg. covered tanks, LIMONENE added manually to tanks)
   - Closed (fully sealed process including automated addition of LIMONENE to tanks)
3. How long does one batch take (if it is batch process)?
4. Any heating is involved?
5. Sample taking? How?
6. Decanting process (automated or manual? How to put cap on container?)

3. How is the equipment used during formulating LIMONENE products cleaned AND how you dispose any cleaning residues?

____

____

____

____

4. Please describe numbers and activities of workers formulating LIMONENE products.

Number	Description of Work	Hrs/day	Days/yr

5. Please describe the engineering controls that are in place to reduce exposure of workers to LIMONENE eg. exhaust ventilation, general dilute ventilation.

Process/Activity	Engineering Controls	Year installed

6. **Please give details of the personal protective equipment used by workers. eg type of gloves, goggles, respirators, protective clothing.**

Process/Activity	Personal Protective Equipment	Type

7. **Are any other precautions taken to reduce exposure of workers to LIMONENE?**

Limited access to area of use, Written procedures for safe use,

Special labeling or placarding. Other________________________

8. **Has atmospheric monitoring been conducted to determine levels of LIMONENE in the workplace?** Yes No

If yes, please provide details of monitoring eg. testing equipment, methods, duration, and results.

____

____

____

9. **Have there been any incidents involving spillage of LIMONENE or LIMONENE products at your workplace AND how they are cleaned?**

Yes No

If yes, please give details:

____

____

____

10. Are you aware of any adverse health effects (eg. skin, eye and respiratory irritation) experienced at your workplace due to exposure and/or spillage of LIMONENE or LIMONENE products?

Yes No

If yes, please give details:

____

____

____

Environmental Effects

11. Is there any LIMONENE waste generate during formulation?

Yes No

If yes, what type?

____

12. What methods do you use to dispose LIMONENE waste?

Blending with other products and re-use

Evaporation to atmosphere

Licensed discharges

Incineration eg. boiler fuel (Cont.)

Send to recycler

Waste collection

Other (please specify)______________________________

13. Please indicate how you handle LIMONENE or LIMONENE products empty containers.

Rinse and/or re-use Return to supplier

Sell to drum recycler Send to landfill

Other (please specify) ______________________________

Thank You for Responding to the Survey!!

Limonene Survey Form 4

This questionnaire applies to companies that use LIMONENE or product(s) containing LIMONENE.

---

1. Product details (for caller to fill):

Product Name	% limonene	Typical end uses

2. Please describe how you use LIMONENE (ie. direct use of raw material) or LIMONENE products ie. details of processes.

Hints:
1. What industry sector is your company in? eg. automative, building, mining, cleaning etc.
2. Do you dilute the product before use? If yes, how (with water?) and in what ratio?
3. How to apply the product - manually? with aid of tools or equipment? automatically?
4. How long does it take each time using the product?
5. How often do you use the product?
6. Roughly estimate how much limonene or limonene products are used per month.

____

____

____

____

____

3. How is the equipment used during using LIMONENE or LIMONENE products cleaned AND how you dispose any cleaning residues?

____

____

____

____

4. **Please describe numbers and activities of workers using LIMONENE or LIMONENE products.**

Number	Description of Work	Hrs/day	Days/yr

5. **Please describe any engineering controls that are in place to reduce exposure of workers to LIMONENE or LIMONENE products eg. exhaust ventilation, general dilute ventilation.**

Process/Activity	Engineering Controls	Year installed

6. **Please give details of the personal protective equipment used by workers during use, eg type of gloves, goggles, respirators, protective clothing.**

Process/Activity	Personal Protective Equipment	Type

7. **Are any other precautions taken to reduce exposure of workers to LIMONENE or LIMONENE products?**

Limited access to area of use, Written procedures for safe use,

Special labeling or placarding. Other________________________

8. **Has atmospheric monitoring been conducted to determine levels of LIMONENE in the workplace?** Yes No

If yes, please provide details of monitoring eg. testing equipment, methods, duration, and results.

____

____

____

9. **Have there been any incidents involving spillage of LIMONENE or LIMONENE products at your workplace AND how they are cleaned?**

Yes No

If yes, please give details:

____

____

____

10. **Are you aware of any adverse health effects (eg. skin, eye and respiratory irritation) experienced at your workplace during use?**

Yes No

If yes, please give details:

____

____

____

Environmental Effects

11. Is there any LIMONENE or LIMONENE product waste generate during use?

Yes No

If yes, what type?

____

12. What methods do you use to dispose LIMONENE or LIMONENE product waste?

Blending with other products and re-use

Evaporation to atmosphere

Licensed discharges

Incineration eg. boiler fuel (Cont.)

Send to recycler

Waste collection

Other (please specify)______________________________

13. Please indicate how you handle LIMONENE or LIMONENE products empty containers.

Rinse and/or re-use

Return to supplier

Sell to drum recycler

Send to landfill

Other (please specify) ______________________________

Thank You for Responding to the Survey!!

If you have any queries about the survey or any of the questions, please contact:

Dr Jun Zhang

Phone 02 9577 9577
Fax: 02 9577 9465
Email: jun.zhang@nicnas.gov.au

Appendix 2

Input to the EASE Modelling

Input to the EASE modelling for estimation of inhalational exposure level (at 20 °C with a vapour pressure of 0.19 kPa).

Process	Use pattern	Control pattern	Result(ppm)
Manufacture	Closed, no system breaching	Full containment	0-0.1
Formulation	Non-dispersive	LEV	0.5-1
	Non-dispersive	Segregation	3-5

Input to the EASE modelling for estimation of dermal exposure level.

Process	Use pattern	Control pattern	Result ($mg/cm^2/d$)
Manufacture	Closed, no system breaching	No direct handling	Very low
Formulation	Non-dispersive	Intermittent direct handling	0.1-1

Closed system	The substance remains within the reactor or is transferred from vessel to vessel through closed pipework. Where breaching of the system occurs, the system is no longer considered to have full containment.
Non-dispersive use	Processes in which substances are used in such a way that only certain group of workers, come into contact with theses chemicals. procedures are normally worked out to achieve adequate control of exposure commensurate with risk.
LEV (local exhaust ventilation)	LEV removes a substance at the point of origin or generation. EASE modelling assumes that it is appropriate for the purpose and operating at or about its design effectiveness.
Segregation	Separating the worker from the substance by distance, typically a few metres from the source of exposure.
Direct handling	The worker handles the substance directly without precautions. The effect of personal protective equipment needs to be judged separately.

Appendix 3

Health effects of limonene or limonene products reported by formulators and users in Australia

Percentage of limonene in product used	Industry sector or use	Effect reported
100%	Histology lab	Headache after using it, even under a hood (extraction). One worker experienced eye irritation or allergic conjunctivitis.
100%	Industrial cleaning of containers	Isolated incident of minor skin irritation when used without PPE.
100%	Cleaning - aircraft components	A worker experienced dermatitis, due to his poor work practices, and is now sensitive to many chemicals.
100%	Gemmology	Strong odour noticed especially when used under microscope, because of heat of microscope light.
100%	Wax removal from surfaces in beauty therapy	Drying of skin on hands if gloves not worn.
30%	Graffiti removal	Sometimes workers become tired of strong odour. Some drying of skin if gloves are not worn.
20%	Textile scouring	No adverse health effects experienced, but when vessel was opened the strong smell could make some people light-headed.
12%	Cleaning – product for shower recesses, benchtops, diluted to wet mop heavy traffic areas	Atomisation of product caused problems for some people – wheezing or breathing problems, coughing.
5%	Cleaning – general purpose for drains, septic tanks etc	Dries skin out.

Appendix 4

Sample Material Safety Data Sheet for *d*-Limonene

Date of issue	22 January 2002	Page	1	of Total	8

***d*-Limonene is classified as hazardous according to the National Occupational Health and Safety Commission's *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(1994)].**

Company Details

Company name		
Address		
	State	Postcode
Telephone number	Emergency telephone number	

Identification

Product Name
d-Limonene

Other names
cyclohexene, 1-methyl-4-(1-methylethenyl)-,(*R*)
(*R*)-*p*-mentha-1.8-diene
(+)-Limonene
(R)-4-Isopropenyl-1-methyl-1-cyclohexene
Carvene
Terpene hydrocarbons NOS (*d*-Limonene) (shipping name)

Manufacturer's product code

UN Number
UN 2319

Dangerous goods class and subsidiary risk
Class 3, Packing Group III

Hazchem code
3[Y]

Poisons Schedule Number
None allocated

Use
Solvent (various applications), fragrance ingredient

Physical description and properties

Appearance
Colourless liquid

Boiling Point
176°C

Freezing Point
-75⁰C

Vapour pressure
0.19 kPa at 20⁰C

Specific Gravity
relative density (water= 1) 0.84

Flashpoint
48°C (closed cup)

Flammability Limits
0.7% to 6.1%

Solubility in water
13.8 mg/L at 25⁰C

Other properties

Odour: Characteristic citrus odour
Vapour density: 4.7 (relative to air = 1)
Evaporation Rate: 5.8 (relative to diethyl ether = 1)
Partition Coefficient: log Pow = 4.23

Reactivity: Autoxidation facilitated by light and air.
Autoignition temperature: 237⁰C

Ingredients/impurities

Chemical entity	CAS Number	Proportion
d-Limonene	5989-27-5	> 95%
Antioxidant (if included)		
Impurities		to 100%

Health hazard information

HEALTH EFFECTS

Acute

Inhalation: Vapour or mists may be irritating to respiratory system. Readily absorbed through inhalation. Strong odour causes discomfort to some people.

Skin: Skin irritant. Can be absorbed through skin.

Eye: Liquid and vapour may cause eye irritation.

Swallowed: Low acute toxicity in animal studies. Ingestion of 20 g caused diarrhoea, painful constrictions and proteinuria in volunteers. Aspiration of limonene may cause lung damage.

Chronic

Skin: There is animal and human evidence of sensitisation by skin contact with oxidised limonene.

Inhalation: There are limited data on the potential for respiratory irritation or sensitisation.

Swallowed: Kidney tumours are induced by limonene in male rats, but are not considered relevant to humans. Liver effects are found in animal studies.

FIRST AID

Inhalation: Remove from exposure. Keep warm and at rest until fully recovered. If there is respiratory distress or aspiration has occurred, seek medical treatment.

Skin: Remove contaminated clothing, rinse skin with water and then wash skin with water and soap.

Eye: Rinse immediately with plenty of water for several minutes. Seek medical treatment if symptoms occur.

Swallowed: Do not induce vomiting, as aspiration may occur and cause lung damage. Give a glass of water. If poisoning occurs, contact a doctor or Poisons Information Centre. Phone 13 1126.

First aid facilities: Safety shower and eye wash basin desirable.

ADVICE TO DOCTOR

Treat symptomatically. No specific antidote.

Precautions for use

EXPOSURE STANDARD

No occupational exposure standard has been assigned for limonene by the National Health and Safety Commission (formerly Worksafe Australia). Other exposure standards are tabulated below.

Country or body	8 h TWA	STEL
Norway (OEL)	25 ppm	-
Sweden	25 ppm	50 ppm
AIHA	30 ppm	

OEL = Occupational exposure limit
TWA = Time weighted average
AIHA = American Industrial Hygiene Association
STEL = short-term (15 min) exposure limit

ENGINEERING CONTROLS

Use only with adequate ventilation. Local exhaust ventilation or flameproof fume cupboards may be necessary for some operations.

Use of closed or semi-closed processes eg lidded tanks, can reduce exposure.

PERSONAL PROTECTION

Use suitable protective clothing to avoid skin contact. Gloves, overalls, aprons, face-shields, goggles or safety glasses and boots may be used. Use manufacturers' recommendations when selecting gloves, as some glove materials are unsuitable for use with limonene.

If necessary, use respirator with organic vapour filter to avoid breathing vapours in confined spaces and in places with limited ventilation.

Page 5 of Total 8

FLAMMABILITY

Flammable liquid. Vapour may form explosive mixtures with air above the flash point of 48^{0}C. Avoid exposure to sources of ignition or open flame. Avoid using in a confined space or generating mists or vapours. May accumulate static charge by flow or agitation. Vapour is heavier than air and may collect in drains or other low areas. Electrically ground all drums, transfer vessels, hoses and piping.

Rags or other combustible material wet or soaked in limonene may autoxidise, generating heat and igniting spontaneously. Used oily rags should be collected regularly and either soaked in water or stored in closed metal containers.

If limonene containing oxidation products is concentrated eg by distillation, explosive levels of peroxides may be formed. Do not distil limonene that may contain peroxides.

Safe handling information

STORAGE and TRANSPORT

Limonene is covered by the Australian Dangerous Goods Code and is a Class 3 (flammable liquid), Packing Group III. Packing Group III indicates lower hazard for flammability as flash point is higher than ambient temperature.

Must be stored in flammable goods stores complying with Commonwealth, State or Territory regulations. Do not weld or cut empty containers.

To avoid oxidation, store in a cool place and out of direct sunlight in full well-sealed containers. Comply with manufacturer's shelf life recommendations, as limonene is known to oxidise.

SPILLS and DISPOSAL

Spills:

Use protective gloves to avoid skin contact. Ventilate area thoroughly and wear a respirator if necessary to minimise inhalation. Eliminate any source of ignition and do not smoke.

Small spills can be wiped up. Rags or other combustible material wet or soaked in limonene may autoxidise, generating heat and igniting spontaneously. Used oily rags should be collected regularly and either soaked in water or stored in closed metal containers.

Large spills should be absorbed by dirt, sand or other suitable absorbents for disposal.

Disposal:

Do not hose spills down drains, sewers or waterways. *d*-Limonene may be toxic to aquatic organisms. Move leaking containers to well ventilated area.

Contact your local waste disposal authority for advice, or pass to a licensed waste disposal company for disposal.

FIRE/EXPLOSION HAZARD

Limonene is a flammable liquid. Vapour may form explosive mixtures at or above 48^0C.

Liquid can float on water and may possibly travel to distant locations and/or spread fire.

Vapour is heavier than air and may spread along ground and collect in low areas.

Carbon monoxide and carbon dioxide may be released in a fire involving limonene.

Hazchem code: 3[Y]

Fire fighting: wear self contained breathing apparatus (SCBA) and complete protective clothing.

Extinguishing media

Dry chemical powder, foam, polymer foam, water spray or fog*.

- Water may be ineffective on fire. However water spray may be used to extinguish fires, because limonene can be cooled below its flash point. Water spray can be used to absorb heat, keep containers cool and protect exposed material. If a leak or spill has not ignited, use water spray to disperse the vapours and to protect personnel attempting to stop a leak. Water spray may be used to flush spills away from exposures.

Page 7	of Total 8

Other information

Toxicological Information

Acute (oral) LD50	4.4/5.1 g/kg bw (rat m/f)
	> 5 g/kg bw (rabbit)
	5.6/6.6 g/kg bw (mouse m/f)

Environmental Data

Acute toxicity	96 h LC50 ≤ 1 mg/L (fish)
	48 h EC50 ≤ 1 mg/L (Daphnia)
	72 h IC50 ≤ 1 mg/L (algae)

Classification
X_i Irritant
N Dangerous for the environment

Risk phrases:
R10 Flammable
R38 Irritating to skin
R43 May cause sensitisation by skin contact

Safety phrases:
S2 Keep out of reach of children
S24 Avoid contact with skin
S37 Wear suitable gloves

Environmental Risk phrases:
R50 Very toxic to aquatic organisms
R53 May cause long term adverse effects in the aquatic environment

Environmental Safety phrases:
S60 This material and/or its container must be disposed of as hazardous waste
S61 Avoid release to the environment. Refer to special instructions/safety data sheet.

Further information

For further information see the NICNAS (National Industrial Chemicals Notification and Assessment Scheme) assessment report on limonene as a priority existing chemical. The full report can be downloaded from http://www.nicnas.gov.au/publications/car/pec/pecindex.htm

Contact Point

Contact name	Telephone number

Position title

Address

State | Postcode | Country

Appendix 5

DRAFT UNPUBLISHED ANALYTICAL PROCEDURE FOR PEROXIDE VALUE

FRAGRANCE MATERIALS ASSOCIATION (FMA)

FMA Instrumental Analysis and Specifications Committee

PEROXIDE VALUE

<u>Definition</u>

"Peroxide value = millimoles peroxide/liter"

A. Reagents:

1. Potassium Iodide, saturated aqueous solution, (freshly prepared).
2. Solvent Mixture 1: Glacial Acetic Acid: Chloroform (3:2).
3. Solvent Mixture 2: Glacial Acetic Acid: Cyclohexane (3:2).
4. Indicator: (Starch Solution or Thyodene)
5. 0.1N Sodium Thiosulfate, volumetric solution (v.s) (0.1N $Na_2S_2O_3$).
6. 0.01N Sodium Thiosulfate, volumetric solution (v.s.) (0.01N $Na_2S_2O_3$). (Prepare Fresh Monthly)
   a. Prepare by pipetting 10.0 ml. of 0.1N $Na_2S_2O_3$ into a 100 ml. volumetric flask and diluting to volume with distilled water.
7. Benzoyl Peroxide: 97% minimum (Aldrich Chemical). If 97% Benzoyl Peroxide is not available, any suitable peroxide may be used as a substitute so long as the concentration is taken into consideration in the calculations.
8. Methanol (Methyl Alcohol), Reagent grade

PROCEDURAL NOTE 1: There are laboratories which do not permit the use of chlorinated solvents. Two solvent systems (Reagents 2 and 3) are provided to address this concern.

PROCEDURAL NOTE 2: Use 0.1N $Na_2S_2O_3$ when expected peroxide values are over 20 mmol/L.

Use 0.01N $Na_2S_2O_3$ when expected peroxide values are less than 20 mmol/L.

PROCEDURAL NOTE 3: This procedure may be performed using mechanical or manual mixing. When using mechanical mixing, a 250 ml. Erlenmeyer flask can be used. When manual mixing and shaking is performed, a 250 ml. Iodine flask is used.

PROCEDURAL NOTE 4: To assure the accuracy of the data, the appropriate standards (as prepared in section D) should be analyzed concurrently with the samples being tested.

B. Procedure:

1. Transfer 10.0 ml. of sample and 50 ml. of the appropriate solvent mixture into the flask and stir at a slow speed or gently shake.
2. Add 1.0 ml. of saturated potassium iodide solution and stir at a slow speed or gently shake for 1 minute.
3. Add 100 ml. of distilled water and 1 ml. starch solution or 0.1 g of Thyodene indicator.
4. Mix aggressively (high speed stirring) or shake well and immediately titrate with 0.1N or 0.01N $Na_2S_2O_3$ from a purple to a slightly yellow or colorless endpoint. (Note: these colors may be affected by the initial color of the test material).

C. Calculation

Peroxide Value (Millimoles Peroxide/Liter) = ml. $_{(titrant)}$ × N $_{(titrant)}$ × 50

Note: Peroxide values may also be calculated as milliequivalents of peroxide. For example, the AOCS method used for fatty oils calls for a weighed sample and calculation as milliequivalents of peroxide per 1,000 grams of sample. Values calculated thus will be approximately twice those calculated by the FMA method.

D. Preparation of Benzoyl Peroxide Standards:

1. Standard 1: (approx 2.0 mmol/L)

   a. Accurately weigh approximately 0.005g. of Benzoyl Peroxide into a 10 ml. volumetric flask and add approximately 9 ml. of methanol. Mix until dissolution is complete (Gently warm if necessary). If the solution is warmed, cool to room temperature and dilute to volume with Methanol. Mix well. Calculate concentration as per step 4.

2. Standard 2.: (approx. 6.0 mmol/L)

   a. Accurately weigh approximately 0.015 g. of Benzoyl Peroxide into a 10 ml. volumetric flask and add aprroximately 9 ml. of methanol. Mix until dissolution is complete (Gently warm if necessary). If the solution is warmed, cool to room temperature and dilute to volume with Methanol. Mix well. Calculate concentration as per step 4.

3. Standard 3.: (approx. 18.0 mmol/L)

   a. Accurately weigh approximately 0.045g of Benzoyl Peroxide into a 10 ml. volumetric flask and add approximately 9 ml of methanol. Mix until dissolution is complete (Gently warm if necessary). If the solution is warmed, cool to room termperature and dilute to volume with Methanol. Mix well. Calculate concentration as per step 4.

4. Calculation of standard concentration:

   a. Wt.std. (g.) $_{(Adjusted)}$ × 100 ÷ 0.2423 = mmol/L

1. wt.std (g.) (Adjusted) = (wt of standard × percent of standard) / 100

5. Calculation of mmol/L peroxide in standard

a. Millimoles Peroxide / Liter = ml. $_{(titrant)}$ × N $_{(titrant)}$ × 50

NOTE 1: No more than 5% error should be found between the calculated and titrated values. Methanol can be run as a blank.

NOTE 2: The committee recommends that the peroxide analyses should be validated by running the appropriate standards concurrently with the sanples.

DISCLAIMER FOR METHOD FOR DETERMINATION OF PEROXIDES:

This method is comparable to other methods used in the scientific community employing similar chemistry. However, after collaborative testing and comparisons of data, the FMA Instrumental Analysis and Specifications Committee has concluded that there may be competing reactions inherent in certain materials which may complicate the endpoint and thus can affect the accuracy of the method. (Note: This phenomena has also been observed using comparable procedures). It is recommeded that replicate analyses be performed.

References

ABS (Australian Bureau of Statistics) (2000) 1996 Census of Population and Housing – Basic Community Profile. Canberra, Commonwealth of Australia. Available from the following URL: <http://www.abs.gov.au/ausstats/abs%40.nsf/7c5e80b14f6c2434ca2568b80016b686/a9399840538865f7ca256ab6001bb7d8!OpenDocument>, Accessed 2002 Mar 25.

ABS (Australian Bureau of Statistics) (2002) 4610.0 Water Account for Australia 1993-94 to 1996-97, Embargo: 11:30 Am (Canberra Time) 03/05/2000. http://www.abs.gov.au/ausstats/abs@.nsf/0/A7F8AE8188119911CA2568D40004EAF7?Open, Accessed 2002 Mar 21.

AIHA (American Industrial Hygiene Association), (1993) Workplace environmental exposure level guide: d-Limonene.

Anderson BE, Zeiger E, Shelby MD, Resnick MA, Gulati DK, Ivett JL & Lovedays KS (1990) Chromosome aberration and sister chromatid exchange test results with 42 chemicals. Environmental and Molecular Mutagenesis, **16**: 55-137.

Anundi H, Langworth S, Johanson G, et al. (2000) Air and biological monitoring of solvent exposure during graffiti removal. Arch Occup Environ Health, **73**: 561-569.

Ariyoshi T, Arakaki M, Ideguchi Y, Noda K & Ide H (1975) Studies on the metabolism of d-limonene (p-mentha-1,8-diene). III. Effects of d-limonene on the lipids and drug metabolizing enzymes in rat livers. Xenobiotica, **5**: 33-38.

Austin CA, Shephard EA, Pike SF, Rabin BR & Phillips IR (1988) The effect of terpenoid components on cytochrome P-450 levels in rat lever. Biochemical Pharmacology, **37**: 2223-2229.

Bagley DM, Gardner JR, Holland G, Lewis RW, Regnier J-F, Stringer DA & Walker AP (1996) Skin irritation: Reference Chemicals Data Bank. Toxicology in Vitro, **10**: 1-6.

Beerman H, Fondé GH & Callaway JL (1938) Citrus fruit dermatoses. Archives of Dermatology and Syphilology (Chicago), **38**(225-234).

Birmingham DJ, Campbell PCJ, Doyle HN & McDonald JM (1951) Investigation of occupational dermatoses in the citrus fruit canning industry. Archives of Industrial Hygiene and Occupational Medicine, **3**: 57-63.

Cachao P, Menezes Brandao F, Carmo M, Frazao S & Silva M (1986) Allergy to oil of turpentine in Portugal. Contact Dermatitis, **14**: 205-208.

Cal K, Janicki S & Sznitowska M (2001) In vitro studies on penetration of terpenes from matrix-type transdermal systems through human skin. International Journal of Pharmaceutics, **224**(1-2): 81-88.

Calnan CD (1979) Allergy to dipentene in paint thinner. Contact Dermatitis, **5**(123-124).

CCOHS (Canadian Centre for Occupational Health and Safety) CHEMINFO [Chemical Profiles for d,l-Limonene, d-Limonene and l-Limonene] [Online]. <http://ccinfoweb.ccohs.ca/cheminfo/> Accessed 5/10/2001.

Chan CC, Vainer L, Martin JW & Williams DT (1990) Determination of organic contaminants in residential indoor air using an adsorption-thermal desorption technique. J. Air Waste Manage. Assoc., **40**: 62-67.

Clark N, Monioudis H & Selikoff IJ (1997) An assessment of metal maintenance workers' solvent exposures. <http://www.cdc.gov/niosh/elcosh/>. Accessed 22/01/2001.

Clegg RJ, Middleton B, Duncan Bell G & White DA (1980) Inhibition of hepatic cholesterol synthesis and S-3-hydroxy-3-methylglutaryl-CoA reductase by mono and bicyclic monoterpenes administered in vivo. Biochemical Pharmacology, **29**: 2125-2127.

Connor TH, Theiss JC, Hanna HA, Monteith DK & Matney TS (1985) Genotoxicity of organic chemicals frequently found in the air of mobile homes. Toxicology Letters, **25**: 33-40.

Cronin E ed (1980) Contact dermatitis. London, Churchill Livingstone, pp532-534.

Crowell PL (1999) Prevention and Therapy of Cancer by Dietary Monoterpenes. J. Nutr., **129**(3): 775S-778S.

Crowell PL, Elegbede JA, Elson CE, et al. (1992) Human metabolism of orally administered d-limonene. Proceedings of the American Association for Cancer Research, **33**: 524.

Dapson JC & Dapson RW (1995) Hazardous Materials in the Histopathology Laboratory: Regulations, Risks, Handling & Disposal, as quoted at http://www.histosearch.com/histonet/Mar00/RE.HistoclearA.html.

Deutsche Forschungsgemeinschaft (2000) List of MAK and BAT values 2000: maximum concentrations and biological tolerance values at the workplace. Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. Report No. 36. Weinheim: Wiley-VCH.

Dheri S (1998) Fire: organizing for fire protection. In: Stellman JM ed. Encyclopaedia of occupational health and safety, 4th ed., vol. 2, pt VI. Geneva, International Labour Office.

Dietrich DR & Swenberg JA (1991) The presence of a2μ-globulin is necessary for d-limonene promotion of male rat kidney tumors. Cancer Research, **51**: 3512-3521.

Eller PM & Cassinelli ME eds (1994) NIOSH manual of analytical methods (4th ed). Cincinatti, Ohio, U.S. Dept of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety & Health, Division of Physical Sciences & Engineering.

European Commission (1996) Technical guidance document in support of Commission Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation (EC) No 1488/94 on risk assessment for existing substances Part 1. Office for Official Publications of the European Communities, Luxembourg.

European Commission (1998) Commission Directive 98/73/EEC of 18 September 1998 adapting to technical progress for the 24th time Council Directive 67/548/EEC on the approximation of the laws, regulations administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities, **L 305**(16/11/98): 1-2, 12-13.

European Union. Scientific Committee for Cosmetic Products and Non-food Products Intended for Consumers (SCCNFP) (2000) Position paper on the phenomenon of quenching. Adopted by the SCCNFP 17/2/00: 11 pp.

European Union. Scientific Committee for Cosmetic Products and Non-food Products intended for Consumers (SCCNFP) (2001) Opinion concerning an initial list of perfumery materials which must not form part of cosmetic products except subject to the restrictions and conditions laid down. Adopted by the SCCNFP 25/9/01, SCCNFP/0392/00, final.

Evans DL, Miller DM, Jacobsen KL & Bush PB (1987) Modulation of immune responses in mice by d-limonene. Journal of Toxicology and Environmental Health, **20**: 51-66.

Fahrig R (1984) Genetic mode of action of cocarcinogens and tumor promoters in yeast and mice. Molecular & General Genetics, **194**: 7-14.

Falk A, Fischer T & Hagberg M (1991) Purpuric rash caused by dermal exposure to *d*-limonene. Contact Dermatitis, **25**: 198-199.

Falk A, Gullstrand E, Lof A & Wigaeus Hjelm E (1990) Liquid/air partition coefficients of four terpenes. British Journal of Industrial Medicine, **47**: 62-64.

Falk-Filipsson A, Lof A, Hagberg M, Wigaeus Hjelm E & Wang Z (1993) d-Limonene exposure to humans by inhalation: uptake, distribution, elimination, and effects on the pulmonary function. Journal of Toxicology and Environmental Health, **38**: 77-88.

Fewings J (2001) Personal communication to R. Sager, NICNAS.

Finnish Institute of Occupational Health (2001) Finnish Occupational Exposure Limits 2000: Unofficial translation, <http://www.occuphealth.fi/ttl/projekti/htp/english/>. Accessed 20/12/01.

Fischer A ed (1986) Contact dermatitis (3rd ed.). London, Churchill Livingstone, pp 421-422, 582-583.

Florin I, Rutberg L, Curvall M & Enzell CR (1980) Screening tobacco smoke constituents for mutagenicity using the Ames test. Toxicology, **18**: 219-232.

FORS (Federal Office of Road Safety) (1998) Australian code for the transport of dangerous goods by road and rail (ADG Code), 6th ed. Canberra, AGPS.

Forsberg K & Mansdorf SZ (1997) Quick selection guide to chemical protective clothing, third ed. NY:Van Nostrand Reinhold.

Gangolli S ed (1999) The dictionary of substances and their effects (2nd edition). Cambridge, Royal Society of Chemistry.

Godwin DA & Michniak BB (1999) Influence of Drug Lipophilicity on Terpenes as Transdermal Penetration Enhancers. Drug Development and Industrial Pharmacy, **25**(8): 905-915.

Grief N (1967) Cutaneous safety of fragrance material as measured by the Maximization Test. American Perfumer and Cosmetics, **82**: 54-57.

Guin J, Mayer B, Drake R & Haffley P (1984) The effect of quenching agents on contact urticaria caused by cinnamic aldehyde. Journal of the American Academy of Dermatology, **1**: 45-51.

Haag KH(1986) Effects of herbicide application on mortality and dispersive bahaviour of the water hyacinth weevils, *Neochetina eichomiae* and *Neochetina bruchi* (Coleoptera: Curculionidae). Environmental Entomology, **15**: 1192-1198.

Haworth SH, Lawlor T, Mortelmans K, Speck W & Zeigor E (1983) *Salmonella* metagenicity test results for 250 chemicals. Environmental Mutagenesis, **1**: 3-142.

HSDB Hazardous Substances Data Bank [Online]. Compiled by the National Library of Medicine, Bethesda, Maryland <http://tomescps.com/>. Englewood, Colorado, MICROMEDEX. Accessed 31/05/2001.

IARC (1993) Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. Lyon, International Agency for Research on Cancer, pp. 135-162 (IARC Monographs on the evaluation of carcinogenic risks to humans, vol. 56).

IARC (1999) Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances. Lyon, International Agency for Research on Cancer (IARC Monographs on the evaluation of carcinogenic risks to humans, vol. 73)

Igimi H, Hisatsugu T & Nishimura M (1976) The use of d-limonene preparation as a dissolving agent of gallstones. Digestive Diseases and Science, **21**: 926-939.

Igimi H, Nishimura M, Kodama R & Ide H (1974) Studies on the metabolism of d-limonene (p-mentha-1,8-diene). I. The absorption, distribution and excretion of d-limonene in rats. Xenobiotica, **4**: 77-84.

Igimi H, Tamura R, Toraishi K, et al. (1991) Medical dissolution of gallstones. Clinical experience of *d*-limonene as a simple, safe and effective solvent. Digestive Diseases and Science, **36**: 200-208.

Inoue S & Yamaguchi H (2000) Systematic absorption of essential oils in mice by holistic vapor exposure. Aroma Res., **1**(3): 72-79.

International Fragrance Association Code and Guidelines: Limonene [Online]. <http://www.ifraorg.org/Guidelines.asp> Accessed 17/06/2001.

IPCS (1997) d-Limonene: International Chemical Safety Card 0918. International Programme on Chemical Safety, <http://www.inchem.org/documents/icsc/icsc/eics0918.htm> Accessed 11/01

IPCS (1998) Concise International Chemical Assessment Document No. 5: Limonene. World Health Organization, Geneva.

Janson R & Kristensson J (1991) Sampling and analysis of atmospheric monoterpenes [Report CM-79]. Stockholm, Sweden, Department of Meteorology, Stockholm University.

Josefsson C (1993) Limonene. In: Health effects of selected chemicals. Vol. 2. Nordic Council of Ministers, Nord 29, Copenhagen.

Kanerva RL, Ridder GM, Lefever FR & Alden CL (1987) Comparison of short-term renal effects due to oral administration of decalin or d-limonene in young adult male Fischer-344 rats. Food and Chemical Toxicology, **25**: 345-353.

Karlberg AT, Boman G & Melin B (1991) Animal Experiments on the Allergenicity of d-Limonene - The Citrus Solvent. Ann. Occup. Hyg., **35**(4): 419-426.

Karlberg AT & Dooms-Goossens A (1997) Contact allergy to oxidized d-limonene among dermatitis patients. Contact Dermatitis, **36**: 201-206.

Karlberg AT & Lindell B (1993) Limonene. In: Beije B, Lundberg P, eds. Criteria documents from Nordic Expert Group 1993. Solna, National Institute of Occupational Health, Nordic Council of Ministers,

Karlberg AT, Magnusson K & Nilsson U (1992) Air oxidation of d-limonene (the citrus solvent) creates potent allergens. Contact Dermatitis, **26**: 332-340.

Karlberg AT, Magnusson K & Nilsson U (1994) Influence of an anti-oxidant on the formation of allergenic compounds during auto-oxidation of d-limonene. Ann. Occ. Hyg., **38**(2).

Kasanen JP, Pasanen AL, Pasanen P, Liesivuori J, Kosma VM & Alarie Y (1999) Evaluation of sensory irritation of D^3-carene and turpentine, and acceptable levels of monoterpenes in occupational and indoor environment. Journal of Toxicology and Environmental Health, **56**: 89-114.

Kiefer M, Bresler F, Salisbury S & Pendergrass S (1994) Investigation of d-Limonene use during aircraft maintenance degreasing operations. Applied Occupational and Environmental Hygiene, **9**(5): 303-311.

Klecak G, Geleick H, Noda K & Ide H (1977) Screening of fragrance materials for allergenicity in the guinea pig. I. Comparison of four testing methods. Journal of the Society of Cosmetic Chemists, **28**: 53-54.

Kodama R, Okubo A, Araki E, Noda K, Ide H & Ikeda T (1977a) Effects on development of mouse fetuses and offspring. (Japan) Oyo Yakuri, **13**: 863-873.

Kodama R, Okubo A, Sato K, Araki E, Noda K, Ide H & Ikeda T (1977b) Studies on d-limonene as a gallstone solubilizer (IX). Effects on development of rabbit fetuses and offspring. (Japan) Oyo Yakuri, **13**: 885-898.

Kodama R, Yano T, Furukawa K, Noda K & Ide H (1976) Studies on the metabolism of d-limonene (p-mentha-1,8-diene). IV. Isolation and characterization of new metabolites and species differences in metabolism. Xenobiotica, **6**: 377-389.

Lacy MJ, Kent CR & Voss EW (1987) d-Limonene: an effective vasodilator for use in collecting rabbit blood. Laboratory Animal Science, **37**(4): 485-487.

Langman JM (1995) d-Limonene: Is it a safe, effective alternative to xylene? The Journal of Histotechnology, **18**(2): 131-137.

Larsen ST, Hougaard KS, Hammer M, et al. (2000) Effects of R-(+) and S-(-)-limonene on the respiratory tract in mice. Human and Experimental Toxicology, **19**: 457-466.

Lehman-McKeeman L & Caudill D (1992) Biochemical basis for mouse resistance to hyaline droplet nephropathy: lack of relevance of the a2m-globulin protein superfamily in this male rat specific syndrome. Toxicology and Applied Pharmacology, **112**: 214-221.

Lehman-McKeeman L, Rodriguez PA, Takigiku R, Caudill D & Fey M (1989) d-Limonene induced male rat specific nephrotoxicity: evaluation of the association between d-limonene and a2m-globulin. Toxicology and Applied Pharmacology, **99**: 250-259.

Louhelainen K, Kangas J, Veijanen A & Viilos P (2001) Effect of in situ composting on reducing offensive odors and volatile organic compounds in swineries. AIHAJ, **62**(2): 159-67.

Maisey J & Miller K (1986) Assessment of the ability of mice fed on vitamin A diet to respond to a varitey of potential contact sensitizers. Contact Dermatitis, **15**: 17-23.

Maltzman TH (1991) Anticarcinogenic mechanisms of action of dietary limonene during the initial stage of DMBA-induced mammary carcinogenesis. Dissertation Abstracts International, **51**: 4283B.

Maltzman TH, Cristou M, Gould MN & Jefcoate CR (1991) Effects on monoterpenoids on in vivo DMBA-DNA adduct formation and on phase I heparic metabolizing enzymes. Carcinogenesis, **12**: 2081-2087.

Mensink B, Monforts M, Wijkhuizen-Maslankiewics L, Tibosch H and Linders J (1995) Manual for summarising and evaluation the environmental aspects of pesticides. Report No. 679101022. National Institute of Public Health and Environmental Protection. Bilthoven, The Netherlands.

Mølhave L, Kjærgaard SK, Hempel-Jørgensen A, Juto JE, Andersson K, Stridh G & Falk J (2000) The eye irritation and odor potencies of four terpenes which are major constituents of the emissions of VOCs from Nordic soft woods. Indoor Air, **10**: 315-318.
National Drugs and Poisons Schedule Committee (1998) Essential Oils Working Party - Chairman's final report, NDPSC.

MITI (1992) Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan compiled under the supervision of Chemical Products Safety Division, Basic Industries Bureau, Ministry of International Trade and Industry Japan. Ministry of International Trade and Industry, Chemicals Inspection and Testing Institute.

NATO (North Atlantic Treaty Organization) Military Agency for Standardization (MAS) (1994) Alternative non-ozone depleting solvents/cleaning agents. (Allied Maritime Environmental Protection Publication (AMEPP) 5, prepared by AC/141(SWG/12)WP/1 15 February 94), 18 pp. <http://www.nato.int/ccms/swg12/docs/amepp5a.htm>. Accessed 25/9/01.

NDPSC (National Drugs and Poisons Scheduling Committee) (1998) Standard for the uniform scheduling of drugs and poisons, No. 16. Canberra, Commonwealth Department of Health and Aged Care.

NICNAS (1999) Priority existing chemical No. 4 secondary notification assessment: 2,2-dichloro-1,1,1-trifluoroethane (HCFC-123): full public report. Sydney, National Industrial Chemicals Notification and Assessment Scheme.

Nilsson U, Bergh M, Shao LP, Karlberg A-T (1996) Analysis of contact allergenic compounds in oxidized d-limonene. Chromatographia **42**(3/4): 199-205

Nilsson U, Magnusson K, Karlberg O, Karlberg A-T (1999) Are contact allergens stable in patch test preparations? Investigation of the degradation of d-limonene hydroperoxides in petrolatum. Contact Dermatitis; **40**(3): 127-32.

NIOSH (1989) Health Hazard Evaluation Report No. 87-379, Keebler Company Bakery, Atlanta, Georgia. Cincinatti, Ohio, National Institute for Occupational Safety and Health.

NOHSC (National Occupational Health and Safety Commission) (1994a) National Model regulations for the control of workplace hazardous substances [NOHSC:1005 (1994)]. Canberra, AGPS.

NOHSC (National Occupational Health and Safety Commission) (1994b) National code of practice for the control of workplace hazardous substances [NOHSC:2007 (1994)]. Canberra, AGPS.

NOHSC (National Occupational Health and Safety Commission) (1994c) National code of practice for the labelling of workplace hazardous substances [NOHSC:2012(1994)]. Canberra, AGPS.

NOHSC (National Occupational Health and Safety Commission) (1994d) National code of practice for the preparation of material safety data sheets [NOHSC:2011(1994)].

NOHSC (National Occupational Health and Safety Commission) (1999) Approved criteria for classifying hazardous substances [NOHSC:1008(1999)]. Canberra, AGPS.

NOHSC (National Occupational Health and Safety Commission) (1999a) List of designated hazardous substances [NOHSC:10005(1999)].

NOHSC (National Occupational Health and Safety Commission) (2001) National standard for the storage and handling of workplace dangerous goods [NOHSC:1015(2001)]. Canberra, AGPS.

NOHSC (National Occupational Health and Safety Commission) (2001a) National Code of Practice for the Storage and Handling of Workplace Dangerous Goods [NOHSC:2017(2001)]. Canberra, AGPS.

Norbäck D, Björnsson E, Janson C, Widstrom J & Boman G (1995) Asthmatic symptoms and volatile organic compounds, formaldehyde and carbon dioxide in dwellings. Occup Environ Med, **52**(6): 388-95.

NTP (1990) Toxicology and carcinogenesis studies of d-limonene in F344/N rats and $B6C3F_1$ mice. Springfield, VA, US Department of Health and Human Services, National Institutes of Health, National Toxicology Program (NTP Technical Report No.347).

OECD (1981a) Modified MITI (Ministry of International Trade and Industry, Japan) Test (I). In: OECD guidelines for testing of chemicals: Section 3 - Degradation and accumulation - ready biodegradability (Guideline 301 C, Adopted 17.07.1992),. Paris, Organisation for Economic Co-operation and Development.

OECD (1981b) Simulation test - aerobic sewage treatment: coupled units test. In: OECD guidelines for testing of chemicals : Section 3 - Degradation and accumulation (Guideline 303 A). Paris, Organisation for Economic Co-operation and Development.

OECD (1981c) Acute dermal irritation/corrosion. In: OECD guidelines for testing of chemicals: Section 4 - Health effects (Guideline 404, Adopted 1981, upgraded 1992). Paris, Organisation for Economic Co-operation and Development.

OECD (2001) Harmonised integrated hazard classification system for chemical substances and mixtures. ENV/JM/MONO(2001)6. Paris, France, Organisation for Economic Co-operation and Development.

Ohio EPA (Environmental Protection Agency) (1997) Alternatives for ozone layer-depleting solvents in metal parts cleaning. Fact sheet number 8. <http://www.epa.state.oh.us/opp/solvents/fact8.html> Accessed 25/9/01.

Okabe H, Obata Y, Takayama K & Nagai T (1990) Percutaneous absorption enhancing effect and skin irritation of monocyclic monoterpenes. Drug Design and Delivery, **6**: 229-238.

Opdyke DLJ (1978) Monographs on fragrance raw materials. Food and cosmetics toxicology, Special issue IV, **16**(Suppl): 809.

Oregon DEQ (Department of Environmental Quality) Alternative Cleaning Solvents and Processes. <http://www.deq.state.or.us/programs/p2/Solvint1.htm> Accessed 25/9/01.

OSHA (Occupational Safety and Health Authority) (1994) Limonene - Partially validated method 2036 <http://www.osha-slc.gov/dts/sltc/methods/partial/pv2036/2036.html>, Accessed 31/05/2001.

Pellizzari ED, Hartwell TD, Harris BSHI, Wadell RD, Whitaker DA & Erickson MD (1982) Purgeable organic compounds in mother's milk. Bulletin of Environmental Contamination and Toxicology, **28**: 322-328.

Pienta RJ (1980) Evaluation and relevance of the Syrian hamster embryo cell system. Applied Methods in Oncology, **3**: 149-169.

Research Triangle Institute & U.S. EPA APPaCDA (2001) SAGE Solvent Alternatives Guide <http://clean.rti.org/index.cfm> Accessed 25/9/01.

Rosenberg C, Ruonakangas A, Liukkonen T, Welling I & Jäppinen P (1999) Exposure to monoterpenes in Finnish Sawmills. American Journal of Industrial Medicine, **Supplement 1**: 149-151.

Rosenberg C, Liukkonen T, Kallas-Tarpila T, Ruonakangas A, Ranta R, Nurminen M, Welling I, Jäppinen P (2002) Monoterpene and wood dust exposures: work-related symptoms among Finnish sawmill workers. American Journal of Industrial Medicine, **41**: 38-53.

RTECS (2001) RTECS: Registry of Toxic Effects of Chemical Substances. National Institute for Occupational Safety and Health, Cincinnati, Ohio <http://www.tomescps.com/>, MICROMEDEX, Englewood, Colorado (Volume 48, accessed 7/10/01).

Rycroft RJG (1980) Allergic contact dernatitis from dipentene in honing oil. Contact Dermatitis, **6**: 325-329.

SAA/SNZ (Standards Australia / Standards New Zealand) (1997) Dangerous Goods - Initial Emergency Response Guide SAA/SNZ HB76.

Schenk J, Däbränte Z, Koch H & Stolte M (1980) Untersuchungen zur Geweberträglichkeit von D-Limonen als einem Läsungsmittel chloesterinreicher Gallensteine. Zeitschrift für Gastroenterologie, **18**: 389-394.

Schwartz L (1938) Cutaneous hazards in the citrus fruit industry. Archives of Dermatology, **37**: 631-649.

Smith O, Wade A & Dean F (1969) Uroterpenol, a pettenkofer chromogen of dietary origin and a common constituent of human urine. Journal of Endocrinology, **45**: 17-28.

Standards Australia (1995) AS-2865-1995: Safe working in a confined space. Homebush, NSW, Standards Australia.

Steele VE, Kelloff GJ, Wilkinson BP & Arnold TJ (1990) Inhibition of transformation in cultured rat tracheal epithelial cells by potential chemopreventive agents. Cancer Research, **50**: 2068-2074.

Svedberg U & Galle B (2000) Assessment of terpene levels and workers' exposure in sawmills with long path FTIR. Applied Occupational and Environmental Hygiene, **15**(9): 686-694.

Tsuji M, Fujisaki Y, Arikawa Y, et al. (1975a) Studies on d-limonene, as gallstone solubilizer (IV): Chronic toxicity in dogs. (Japan) Oyo Yakuri, **9**: 775-808.

Tsuji M, Fujisaki Y, Okubo A, Arikawa Y, Noda K, Ide H & Ikeda T (1975b) Studies on d-limonene, as gallstone solubilizer (V): Effects on development of rat fetuses and offspring. (Japan) Oyo Yakuri, **10**: 179-186.

Tsuji M, Fujisaki Y, Yamachika K, et al. (1974) Studies on d-limonene, as gallstone solubilizer (I): General pharmacological studies. (Japan) Oyo Yakuri, **8**: 1439-1459.

UK HSE (2000) EASE (Estimation and Assessment of Substance Exposure) model (version 2.0 for windows). United Kingdom Health and Safety Executive.

US EPA (1994) Reregistration Eligibility Decision (RED) Limonene. US Environmental Protection Agency, Prevention, Pesticides and Toxic Substances (Report No. EPA-738-R-94-034)

Urben PGE (1999) Bretherick's Handbook of Reactive Chemical Hazards 6th Edition, Volume 2, p. 137.

Vigushin DM, Poon GP, Boddy A, et al. (1998) Phase I and pharmacokinetic study of D-limonene in patients with advanced cancer. Cancer Chemother Pharmacol, **42**: 111-117.

von Schafer R & Schafer W (1982) Die perkutane Resorption verscheidener Terpene-Menthol, Campher, Limonen, Isobornylacetat, a-pinen-aus badezusatzen. Drug Research, **32**: 56-58.

Washington State Dept of Ecology (1996) Alternatives to chlorinated solvents: solvent substitution options. Ecology Fact Sheet 96-420. 44pp. Accessed at www.ecy.wa.gov/pubs/96420.pdf 25/9/01.

Watabe T, Hiratsuka A, Osawa N & Isobe M (1981) A comparative study on the metabolism of d-limonene and 4-vinylcyclohex-1-ene by hepatic microsomes. Xenobiotica, **11**: 333-334.

Webb DR, Kanerva LL, Hysell DK, Alden CL & Lehman-McKeeman L (1990) Assessment of the subchronic oral toxicity of d-limonene in dogs. Food and Chemical Toxicology, **28**: 669-675.

Webb DR, Ridder GM & Alden CL (1989) Acute and subchronic nephrotoxicity of d-limonene in Fischer 344 rats. Food and Chemical Toxicology, **27**: 639-469.

York M, Basketter Cuthbert JA & Neilson L (1995) Skin irritation in man for hazard assessment - evaluation of four patch systems. Human and Experimental Toxicology, **14**: 729-734.

Zhang Z, Chen H, Chan KK, Budd T & Ganapathi R (1999) Gas chromatographic-mass spectrometric analysis of perillyl alcohol and metabolites in plasma. Journal of Chromatography B, **728**: 85-95.

Zinkel DF & Russell J (Eds) (1989) Naval stores: production, chemistry, utilization. USA: Pulp Chemicals Association.

Zitko V (2001) Peroxides in Histo-Clear.
<http//preprint.chemweb.com/chemistry/0103001> Accessed 14/8/01.

Order Form

FOR NICNAS PUBLICATIONS

NICNAS

List of Publications	Quantity	Amount
Handbook for Notifiers @ AUD $103.00 each		
Australian Inventory of Chemical Substances (AICS) CD ROM @ $195.00 (Annual subscription with free six-monthly update). **Available within Australia only.**		
Copy/s of Full Public Report/s of the following complete assessments. *Include NICNAS reference number/s* ***(no charge).***		
Full Public Report for Priority Existing Chemical – *Please specify report name* ***(no charge).***		
	Total	$

All prices include postage and packaging within Australia and by SEAMAIL overseas.
For AIRMAIL please include an additional $50.00 per Handbook and $10.00 each for other NICNAS products.

All orders must be accompanied by prepayment in Australian Dollars. Purchase orders NOT accepted.

Overseas only: Please send by AIRMAIL Yes ◯ No ◯

I enclose a cheque/money order payable to: National Occupational Health & Safety Commission.

Drawn on an Australian bank in Australian Dollars for: $

or: Bankcard / Visacard / Mastercard **only.** Card no.

Signature of card holder Expiry date ______

☒ ______ Name of card holder ______

Please ensure you complete this section.

Name of recipient ______ Position ______

Company ______

Address ______

Telephone ( ) ______ Fax ( ) ______

Send this order to: National Occupational Health & Safety Commission, Finance Section
GPO Box 58, Sydney, NSW 2001 Australia

For further information about NICNAS publications please call: (02) 9577 9579 (International +61 2 9577 9579)
Or fax: (02) 9577 9465 (International +61 2 9577 9465)