FOREWORD INTRODUCTION

CITRAL

CAS N°:5392-40-5

SIDS Initial Assessment Report for 13th SIAM

(Switzerland, November 6-9, 2001)

Chemical Name: Citral

CAS No: 5392-40-5

Sponsor Country: Japan

National SIDS Contact Point in Sponsor Country:
Mr. Yasuhisa Kawamura, Ministry of Foreign Affairs, Japan

History:

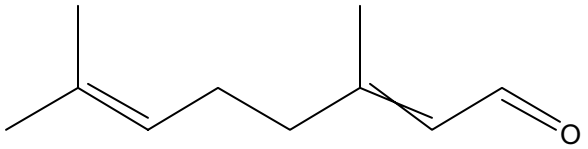
This SIAR was discussed at SIAM11 (USA, January, 2001) at which the human health assessment and its conclusion were accepted. The environmental assessment was revised after SIAM11 and the revised SIAR was discussed and finalised at SIAM13.

Tests:

No testing	( )
Testing (x)	Vapor pressure, log P_{ow}, Water solubility, Hydrolysis and Photolysis, Biodegradation, Environmental fate, Acute toxicity to fish, daphnia and algae, Chronic toxicity to daphnia, Preliminary reproduction toxicity

Comment:

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	5392-40-5
Chemical Name	Citral
Structural Formula	 $C_{10}H_{16}O$

RECOMMENDATIONS

The chemical is currently of low priority for further work.

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

Citral was rapidly absorbed from the gastro-intestinal tract. Much of an applied dermal dose was lost due to its extreme volatility, but the citral remaining on the skin was fairly well absorbed. Citral was rapidly metabolized and excreted as metabolites. Urine was the major route of elimination.

Acute toxicity of this chemical is low in rodents because the oral or dermal LD_{50} values were more than 1000 mg/kg. This chemical is irritating to skin and not irritating to eyes in rabbits. There is some evidence that this chemical is a human skin sensitizer.

Several repeated dose oral studies show no adverse effect of citral at less than 1,000 mg/kg/day exposure and some histological changes in the nasal cavity or forestomach, the first exposure sites, probably due to irritation, at more than 1,000 mg/kg/day. Male and female F344/N rats received microencapsulated citral in feed at concentrations of 0, 0.63, 1.25, 2.5, 5 and 10% (resultant doses: 0, 142, 285, 570, 1,140 and 2,280 mg/kg/day) for 14 days. Minimal to mild hyperplasia and/or squamous metaplasia of the respiratory epithelium was observed in nasal cavity without inflammatory response at 1,140 and 2,280 mg/kg/day of both sexes. The NOAEL was established at 570 mg/kg/day. In an OECD preliminary reproduction toxicity screening test [TG 421], citral was administered to Crj:CD (SD) rats by gavage at doses of 0, 40, 200 and 1,000 mg/kg/day in males for 46 days and in females for 39-50 days including before and through mating and gestation periods and until day 3 of lactation. Squamous hyperplasia, ulcer and granulation in lamina propria were observed in the forestomach at 1,000 mg/kg/day of both sexes. Therefore, the NOAEL for repeated dose toxicity was 200 mg/kg/day for both sexes.

As for reproductive toxicity in the above preliminary reproductive study, no effects were detected in reproductive ability, organ weights or histopathology of the reproductive organs of both sexes, and delivery or maternal behavior. However, body weights of male and female pups were reduced in the 1000 mg/kg group. Therefore, an oral NOAEL for developmental toxicity was 200 mg/kg/day. In a teratogenicity study, SD pregnant rats were exposed to citral by inhalation for 6 hr/day on gestation days 6-15 at mean concentration of 0, 10 or 34 ppm as vapour, or 68 ppm as an aerosol/vapour mixture. Even in the presence of the maternal effects, no significant teratogenicity was noted at 68 ppm. An inhalation NOAEL of teratogenicity was established at 68 ppm (423 mg/m^3).

Seven bacterial reverse mutation studies indicate negative results with and without metabolic activation. As for non-bacterial *in vitro* study, two chromosomal aberration results in Chinese hamster cells are negative however one positive result in sister chromatid exchange is given in the same cells. Additionally, two *in vivo* micronucleus tests in rodents indicate negative results. Based on the above information, the genotoxic potential of citral can be considered to be negative.

A NTP study shows that there was no evidence of carcinogenic activity in male/female rats and male mice but some evidence of malignant lymphoma in female mice (up to 4,000 ppm in feed in rats and up to 2,000 ppm in feed in mice).

Dermal application of citral induces prostate hyperplasia with low severity only in some strains of rats. However, the NTP oral carcinogenicity studies in rats and mice found no evidence of lesions (neoplastic or non-neoplastic) in any male reproductive organ, including the prostate. The health significance of the effects seen in the dermal studies in rats is uncertain due to dramatic strain differences and it is noted that the work has primarily been performed in a single laboratory.

Environment

Citral is readily biodegradable (92%, BOD) and its bioaccumulation potential seems to be low based on Log P_{ow} (2.8-3.0). This chemical has been tested in a limited number of aquatic species. For alga (*Selenastrum capricornutum*), 72 h EC_{50} (biomass) is 5 mg/L and 72 h NOEC (biomass) is 3.1 mg/L. For *Daphnia*, acute toxicity of 10 mg/l (24hEC50, immobilizatio n) and 7 mg (48h-EC50, immobilization), and chronic values of 1.0 mg/L (21 d NOEC, reproduction) have been reported. Only the acute toxicity value has been reported for fish, which is 4.1 mg/L (96 h LC_{50}) for *Oryzias latipes.* A PNEC of 0.01 mg/L for the aquatic organisms was calculated from the chronic toxicity value of *Daphnia* using an assessment factor of 100.

Exposure

Production volume of citral in Japan was 1,200 tonnes in 1990-1999. This chemical is used as a food flavoring and as an intermediate for perfume and vitamin A production. This chemical is a mixture of two geometric isomers, geranial (trans confirmation, approx. 55-70%) and neral (cis confirmation, 35-45%). It is rapidly hydrolyzed at pH 4 (half-life time: Neral: 9.54 days, Geranial: 9.81days) and at pH 9 (half-life time: Neral: 30.1 days, Geranial: 22.8 days), but slowly hydrolyzed at pH 7 (half-life time: Neral: 230 days, Geranial: 106 days). This chemical is classified as "readily biodegradable". A generic fugacity model (Mackey level III) shows that if citral is released to one of the compartments of air, water and soil, it is unlikely to distribute into other compartments. According to a Japanese manufacturer, 1,200 kg/year (estimated) of citral are treated in waste water treatment plants and then released with 5,000 t/year of effluent into a river (flow rate 1.6 $x10^{11}$ t/year). The local predicted environmental concentration (PEC_{local}) is $7.5x10^{-7}$ mg/l, employing a calculation model. The highest exposure to the general populatio n via the environment would be expected through drinking water processed from surface water. The concentration of citral in drinking water is assumed to be less than 7.5 $x10^{-7}$ mg/l. Occupational exposures at production sites may occur by the inhalation and dermal route. The estimated human exposure of a worker who operates the drum filler and does sampling assuming without protective equipment is 0.34 mg/kg/day. However, protective measures i.e. safety glasses and gloves are used during these processes. Therefore, the actual exposure to workers is lower than the estimated value.

NATURE OF FURTHER WORK RECOMMENDED

No recommendation.

FULL SIDS SUMMARY

CAS NO: 5392-40-5		SPECIES	PROTOCOL	RESULTS
PHYSICAL-CHEMICAL				
2.1	Melting Point		Unknown	<- 10 °C
2.2	Boiling Point		Unknown	226-228 °C
2.3	Density		Unknown	0.891-0.897 at 15°C
2.4	Vapour Pressure		OECD TG 104	<130 Pa at 100 °C
2.5	Partition Coefficient (Log Pow)		OECD TG 117	2.8 at 25 °C (neral) 3.0 at 25 °C (geranial)
2.6 A.	Water Solubility		OECD TG 105	590 mg/L at 25 °C
B.	pH			None
	pKa			None
2.12	Oxidation: Reduction Potential			None
ENVIRONMENTAL FATE AND PATHWAY				
3.1.1	Photodegradation			$T_{1/2}$=1.14 years (direct) $T_{1/2}$=2.83 hours (indirect)
3.1.2	Stability in Water		OECD TG 111	neral T1/2=9.54 days (pH 4) T1/2=230 days (pH 7) T1/2=30.1 days (pH 9) geranial T1/2=9.81 days (pH 4) T1/2=106 days (pH 7) T1/2=22.8 days (pH 9)
3.2	Monitoring Data			In air = None In surface water = None In soil/sediment = None In biota = None
3.3	Transport and Distribution		Calculated (Level III Fugacity Model)	(Release 100% to air) Air Water Soil Sediment 97.7% 1.6% 0.7% 0.0% (Release 100% to water) Air Water Soil Sediment 1.7% 97.0% 0.0% 1.3% (Release 100% to soil) Air Water Soil Sediment 0.1% 0.2% 99.7% 0.0%
			(local exposure)	PEC_{local} = 7.5x10^{-7} mg/L
3.5	Biodegradation		OECD TG 301C	Readily biodegradable
3.7	Bioaccumulation			None
ECOTOXICOLOGY				
4.1	Acute/Prolonged Toxicity to Fish	*Oryzias latipes*	OECD TG 203	LC_{50} (96 h): 4.1mg/L
4.2	Acute Toxicity to Aquatic Invertebrates	*Daphnia magna*	OECD TG 202	EC_{50} (24hr, Imm)= 10 mg/L
		Daphnia magna	Other	EC_{50} (48hr)= 7 mg/L

CAS NO: 5392-40-5		SPECIES	PROTOCOL	RESULTS
4.3	Toxicity to Aquatic Plants e.g. Algae	*Selenastrum capricornutum*	OECD TG 201	EC_{50} (72hr)= 5 mg/L NOEC (72hr)= 3.1 mg/L
		Selenastrum capricornutum	Other	EC_{50} (72hr)= 16 mg/L EC_{50} (96hr)= 19mg/L
4.5.2	Chronic Toxicity to Aquatic Invertebrates	*Daphnia magna*	OECD TG 202	EC_{50} (21d, Repro)=1.6 mg/L NOEC (21d, Repro)= 1.0 mg/L
TOXICOLOGY				
5.1.1	Acute Oral Toxicity	Rat	Other (unknown)	LD_{50} = 4,960 mg/kg b.w.
5.1.3	Acute Dermal Toxicity	Rabbit	Other (unknown)	LD_{50} = 2,250 mg/kg b.w.
5.2.1	Skin Irritation	Rabbit	Other (unknown)	Irritating
5.2.2	Eye Irritation	Rabbit	Other (unknown)	Not irritating
5.3	Sensitization	Guinea pig	Other (maximization test)	Sensitizing
5.4	Repeated Dose Toxicity	Rat	Other (microcapsule)	NOAEL = 570 mg/kg/day
		Rat	OECD TG 421	NOAEL = 200 mg/kg/day
5.5	Genetic Toxicity In Vitro			
A.	Bacterial Test (Gene mutation)	*S. typhimurium*	Other (unknown)	- (With metabolic activation) - (Without metabolic activation)
B.	Non-Bacterial In Vitro Test (Chromosomal aberrations)	CHL cells	Other (unknown)	- (With metabolic activation) - (Without metabolic activation)
	(Sister chromatid exchange)	CHL cells	Other (NTP)	+ (With metabolic activation) + (Without metabolic activation)
5.6	Genetic Toxicity In Vivo	Mouse	Other (NTP)	-
5.8	Toxicity to Reproduction	Rat	OECD TG 421	NOAEL = 1,000 mg/kg/day
5.9	Developmental Toxicity/ Teratogenicity	Rat	OECD TG 421	NOAEL = 200 mg/kg/day
		Rat	Other (inhalation)	NOAEL = 423 mg/m^3 No teratogenicity
5.11	Experience with Human Exposure	Human	Other (Patch test)	Not irritating nor sensitising by consumer products

SIDS INITIAL ASSESSMENT REPORT

1. Identity

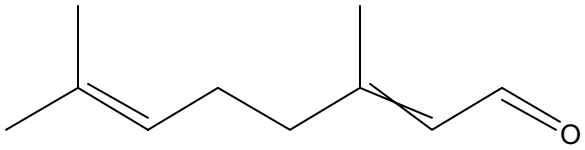
OECD Name: Citral

Synonym: 2,6-Octadienal, 3,7-dimethyl-

CAS Number: 5392-40-5

Empirical Formula: $C_{10}H_{16}O$

Structural Formula:



Degree of Purity: > 95 %

Major Impurities: Unknown

Essential Additives: None

Physical and chemical properties:

	Protocol	Results
Melting Point	Unknown	< - 10 °C
Boiling Point	Unknown	226 – 228 °C
Density	Unknown	0.891 - 0.897 g/cm^3 at 15 °C
Vapour Pressure	OECD TG 104	< 130 Pa at 40 °C
Partition Coefficient (Log Pow)	OECD TG 107	2.8 at 25 °C (neral) 3.0 at 25 °C (geranial)
Water Solubility	OECD TG 105	590 mg/L at 25°C

Citral is a mixture of two geometric isomers, geranial (trans confirmation, approx. 55-70 %) and neral (cis confirmation, 35-45 %).

2. Exposure

2.1 General discussion

The production level of citral in Japan was about 1,200 tonnes/year. Most of this amount was sold and handled in Japan. There is no information about imported volumes of citral. Citral is readily biodegradable (OECD 301C: 92 % after 28 days) and its bioaccumulation potential seems to be low based on Log P_{ow} (2.8-3.0). Citral is rapidly decomposed at pH 4 (half-life time: neral: 9.54 days, geranial: 9.81 days) and 9 (half-life time: neral: 30.1 days, geranial: 22.8 days), but slowly decomposed at pH 7 (half-life time: neral: 230 days, geranial: 106 days). This chemical is hydrolyzed into geranic acid and 6-methyl-5-heptene-2-on. It is also reported that this chemical is converted into 2-formylmethyl-2-methyl-5-(1-hydroxy-1-methylethyl)- tetrahydrofuran under oxygen atmosphere in aqueous acidic condition [B. Grein et al.: 1994].

2.2 Environmental exposure

a. Global exposure

The potential environmental distribution of citral obtained from a generic level III fugacity model under three emission scenarios is shown in Table 1. The results show that if citral is released to one of the compartments of air, water and soil, it is unlikely to distribute into other compartments.

Table 1: Environmental distribution of Citral using a generic level III fugacity model under three emission scenarios

Compartment	Release: 100 % to air	Release: 100 % to water	Release: 100 % to soil
Air	97.7 %	1.7 %	0.1 %
Water	1.6 %	97.0 %	0.2 %
Soil	0.7 %	0.0 %	99.7 %
Sediment	0.0 %	1.3 %	0.0 %

Remarks: Concerning the vapor pressure, a calculated value by QSAR was used for this fugacity calculation (12.1 Pa as a vapour pressure), since we have only the range of this property (< 130 Pa as vapour pressure).

b. Local exposure

According to a Japanese manufacturer, 1,200 kg/year (estimated) of citral are treated in a waste water treatment plant (WWTP) and then released with 5,000 t/year of effluent into a river (flow rate 1.6 x 10^{11} t/year). The local predicted environmental concentration (PEC_{local}) is 7.5 x 10^{-7} mg/l, employing the following calculation model. In this case, elimination factor in the WWTP is estimated to be 90%.

$$PEC = \frac{\text{Amount of release } (1.2 \times 10^{9} \text{ mg/y}) \times (1 - 0.90)}{\text{Volume of effluent } (5.0 \times 10^{6} \text{ l/y}) \times \text{Dilution factor } (1.6 \times 10^{11} \text{ t/y} / 5.0 \times 10^{3} \text{ t/y})} = 7.5 \times 10^{-7} \text{ mg/l}$$

2.3 Consumer Exposure

Citral is used as a food flavor, intermediates for vitamin A and perfume such as ionon. Potential exposure to consumers may exist.

2.4 Exposure via the environment

The highest exposure to the general population via the environment would be expected through drinking water processed from surface water. Based on the physical chemical properties of Citral, a significant removal of during processing is expected. Although PEC_{global} cannot be estimated, the concentration in drinking water is assumed to be less than 7.5×10^{-7} mg/l.

2.5 Occupational Exposure

- Occupational exposures at production sites may occur by the inhalation and dermal route.
- The atmospheric concentration was measured at one production site. The monitored data are shown in Table 2.

Table 2: Available workplace monitoring data for Citral

Occupation (Country)	Activity	Monitoring data	Comment	Source
Citral manufacture (Japan)	Drum filling	0.31-0.56 mg/ m^3	Air samples around the drum were taken by Tenax tubes. Analyzed by purge & trap sampling and GC/FID.	Report from Manufacturer (1987)

- Based on the maximum concentration of 0.56 mg/m3, the EHEinhalation of a worker who operates the drum filler for 4 hours and does sampling for 12 minutes a day without protective equipment is 0.04 mg/kg/day.
- Dermal exposure during sampling was estimated using the EASE modelas non-dispersive, direct handling was 0.1-1 mg/cm2/day. The EHEdermal for 12 minutes sampling work was 0.3 mg/kg/day, assuming both hands were exposed.
- The combined EHE would be 0.34 mg/kg/day. Workers always wear protective gloves and respiratory protective equipment (mask) during these operations.
- No occupational exposure limit for this chemical was located.

3. EFFECTS ON THE ENVIRONMENT

3.1 Toxicity to Aquatic Organisms

Citral has been tested in a limited number of aquatic species. Results are summarized in Table 3. Almost all data shown here were obtained by the Environment Agency of Japan during 1992-1993. The experimental conditions and results were well documented in these studies, although the chemical concentrations in the test medium had not been measured during the course of the experiments. Representatives of all the three trophic levels showed similar sensitivity to this chemical.

Table 3: Summary of effects of Citral on aquatic organisms

Organism	Test duration	Result (mg/L)	Reference
Aquatic plants, e.g. algae			
Green alga (*Selenastrum capricornutum*)	72 h (cl,s)	EC_{50}(Bms) =5 (nc)	Japan EA (1994)
	72 h (cl,s)	NOEC(Bms) =3.1 (nc)	Japan EA (1994)
	72 h	EC_{50} (Bms) = 16	BASF AG
	96 h	EC_{50} (Bms) = 19	BASF AG
Invertebrates			
Water flea (*Daphnia magna*)	24 h (cl,s)	EC_{50} (Imm) =10 (nc)	Japan EA (1994)
	48 h	EC_{50} = 7	BASF AG
	21 d (cl,ss)	EC_{50} (Rep) = 1.6 (nc)	Japan EA (1994)
	21 d (cl,ss)	NOEC(Rep) =1.0 (nc)	Japan EA (1994)
Fish			
Medaka (*Oryzias latipes*)	96 h (cl,ss)	LC_{50} = 4.1 (nc)	Japan EA (1994)

cl = closed system, m = measured concentration, f = flow through, op = open system, s = static, ss = semi-static, nr = Not described for test type, nc = nominal, Bms = biomass, Imm = immobilization, Rep = reproduction, Gro=Growth

3.2 Toxicity to Terrestrial Organisms

There is no available information.

3.3 Other

There is no available information.

3.4 Initial Assessment for the Environment

Citral is readily biodegradable and bioaccumulation potential seems to be low based on Log Pow (2.8-3.0). The lowest acute and chronic data were 4.1 mg/L (Oryzias 96h LC50) and 1 mg/L (Daphnia 21 d NOEC, reproduction), respectively. The predicted no effect concentration (PNEC) of 0.01 mg/L for the aquatic organisms was calculated from the NOEC for Daphnia using an assessment factor of 100, because two chronic data (Daphnia and Selenastrum) were available.

4 HUMAN HEALTH HAZARDS

4.1. Effects on Human Health

a) Toxicokinetics and metabolism

There is some information on metabolism and toxicokinetics of citral.

Orally administrated citral was absorbed rapidly and almost completely from the gastro-intestinal tract in rats and mice [Pillips et al.: 1976, Diliberto: 1988]. Much of an applied dermal dose was lost due to its extreme volatility, but the citral remaining on the skin was fairly well absorbed in rats [Diliberto et al.: 1988].

After a single oral dose, citral was rapidly metabolized and excreted as metabolites, including several acids and a biliary glucuronide in male F344 rats [Diliberto et al.: 1990]. In in vitro metabolism study, citral was not oxidized by aldehyde dehydrogenases (ALDH) from either hepatic mitochondrial or cytosolic fraction prepared from male SD rats [Boyer et al.: 1991]. Citral was readily reduced to the corresponding alcohol by alcohol dehydrogenase (ADH) in cytosolic fraction. Citral may undergo an ADH-mediated reduction or metabolism via other pathways rather than the oxidation by ALDH.

The disposition of [14C] citral was studied in male Fischer rats after iv, po and dermal treatments [Diliberto et al.: 1988]. At 72 hr after treatment, the amount of 14C found in any tissue was a very small percentage (< 2%) of the total dose. The relative amount of radioactivity in all tissues did not change with increasing dose or route of exposure.

Citral was excreted rapidly and most of the administered radioactivity was excreted within 72 hr by the rat and within 120 hr by the mouse after oral administration with [14C] citral [Phillips et al.: 1976]. Urine was major route of elimination, followed by feces, CO2 (via lung) and exhaled volatiles [Diliberto et al.: 1988]. The pattern of elimination was the same after iv or oral exposure in rats. However, after dermal exposure, relatively less of the material was eliminated in the urine and more in the feces, suggesting a role for first-pass metabolism through the skin.

There was no evidence for long-term retention of citral in the body, and it is suggested that any hazard associated with tissue accumulation after prolonged exposure will be minimal [Phillips et al.: 1976]. Repeated exposure to citral resulted in an increase in biliary elimination, without any significant change in the pattern of urinary, fecal, or exhaled excretion [Diliberto et al.: 1988].

b) Acute toxicity

There are many studies on acute toxicity of citral for rats, mice and rabbits, of which none was identified as a key study. However, reassuring is that the data show consistency of results (Table 4). Acute toxicity of citral is likely low because the oral or dermal LD50 values were more than 1,000 mg/kg.

Table 4: Acute toxicity of citral in experimental animals

Route	Animals	Values	Type	References
Oral				
	Rat	6800mg/kg	LD_{50}	BASF AG: 1978
	Rat	4950 mg/kg	LD_{50}	BASF AG: 1957
	Rat	4960 mg/kg	LD_{50}	Jenner et al.: 1979
	Mouse	1440 mg/kg	LD_{50}	BASF AG: 1957
	Mouse	6000 mg/kg	LD_{50}	Biochem. J.: 1940
	Mouse	900 mg/kg	LD_{0}	Le Bourhis et al.: 1973
Dermal	Rat	> 2000 mg/kg	LD_{50}	BASF AG: 1978
	Rabbit	2250 mg/kg	LD_{50}	Moreno: 1979

Human data

There is no available information on humans.

Conclusions:

Acute toxicity of this chemical is low in rodents because the oral or dermal LD50 values were greater than 1000 mg/kg.

c) Repeat dose toxicity

Several oral studies in rats and mice show no adverse effect of citral at less than 1,000 mg/kg for 14 days to 13 weeks exposure and some histological changes in the nasal cavity or forestomach, probably due to irritation, at 1,000 mg/kg and higher dose levels as shown in Table 5 [Hagan et al.: 1967, Dieter et al.: 1993, MHW, Japan: 2002]. However most studies were not conducted under GLP nor using published test guidelines. Hagan's feeding study is not appropriate as a key study because the food might not have kept the indicated citral concentrations due to the high volatility. Among the others, two rat studies, a microcapsule study by Dieter et al. and a gavage study by MHW were identified as the key studies because they were well conducted and reported.

Table 5: Repeated oral dose toxicity of citral

Route	Animal	Period	Doses	NOAEL mg/kg/day	LOAEL mg/kg/day	Toxic effects	Reference
Capsule	Rats	14 days	142 - 2280 mg/kg	570	1140	nasal cavity	Dieter: 1993
Gavage	Rats	14 days	570 - 2280 mg/kg	1140	2280	forestomach	Dieter: 1993
Gavage	Rats	46 days	40 - 1000 mg/kg	200	1000	forestomach	MHW: 2002*
Feed	Rats	13 weeks	83 - 833 mg/kg	833	-	none	Hagan: 1967
Capsule	Mice	14 days	534 - 8550 mg/kg	8550	-	none	Dieter: 1993
Gavage	Mice	14 days	534 - 2137mg/kg	534	1068	forestomach, liver	Dieter: 1993

* an OECD preliminary reproduction toxicity screening test

Male and female F344/N rats received microencapsulated citral in feed because of the high volatility, at concentrations of 0, 0.63, 1.25, 25, 5 and 10 % (resultant doses: 0, 142, 285, 570, 1,140 and 2,280 mg/kg/day) for 14 days [Dieter et al.: 1993]. No clinical signs were detected in all treated groups. Reduction in final body weight was noted in both sexes of 2,280 mg/kg. Histopathological examination revealed minimal to mild hyperplasia and/or squamous metaplasia of the respiratory epithelium in nasal cavity, without inflammatory response at 1,140 and 2,280 mg/kg of both sexes. The NOAEL was established at 570 mg/kg/day.

In an OECD preliminary reproduction toxicity screening test, citral was administered to Crj:CD (SD) rats by gavage at doses of 0, 40, 200 and 1,000 mg/kg/day in males for 46 days and in females for 39-50 days including before and through mating and gestation periods and until day 3 of lactation [MHW, Japan: 2002]. Hematology and serum biochemistry were not examined because of the test guideline and histopathology was conducted only in stomach and reproductive organs.

Any toxic sign in general appearance was not observed. Necropsy revealed a partial thickening of mucosal layer in the stomach of females, and histopathological examination in the forestomach revealed squamous hyperplasia, ulcer and granulation in lamina propria at 1,000 mg/kg of both sexes. Any histological changes in reproductive organs of both sexes were not observed. The NOAEL for repeated dose toxicity was 200 mg/kg/day for both sexes.

As citral is quickly absorbed and metabolized, the toxicity profile depends on administration methods. In Dieter's study [1993], a high stomach concentration given by gavage induced forestomach hyperplasia probably due to irritating activity at the similar doses in rats and mice. However, a dietary administration in microcapsules might produce continuous but lower body burden including stomach during feeding. By this difference, no region in stomach was observed in rats and mice.
Clear species differences were observed in dietary studies. Rats received microencapsulated citral in diet showed minimal to mild hyperplasia and/or metaplasia in respiratory epithelium of nasal cavity at two highest doses. However, mice did not show any regions in nasal cavity at the same levels. Because authors mentioned that the smell of citral at the high concentration might have induced to reject feeding at the beginning of study, the region on nasal cavity in rats could have been due to the direct effect of citral. The reason of no effects in mice is not clear sure but it seems likely that a nasal cavity of rats is generally more sensitive than that of mice.

There is no available information on humans.

Conclusions:
Several repeated dose oral studies show no adverse effect of citral at less than 1,000 mg/kg for 14 days to 13 weeks exposure and some histological changes in nasal cavity or forestomach at the first exposure sites, probably due to irritation, at more than 1,000 mg/kg. The NOAEL for repeat dose toxicity was considered to be the lowest NOAEL of 200 mg/kg/day.

d) Reproduction/developmental toxicity

Reproductive toxicity
The only available data is from the OECD preliminary reproduction toxicity screening test (TG 421) [MHW, Japan: 2002]. This study was well conducted and reported.

In an OECD preliminary reproduction toxicity screening test, citral was administered to Crj:CD(SD) rats by gavage at doses of 0, 40, 200 and 1,000 mg/kg/day in males for 46 days including before and during mating period, and in females for 39-50 days including before and through mating and gestation periods and until day 3 of lactation.

No statistically significant effects were detected in reproductive ability, organ weights or histopathology of the reproductive organs of both sexes, and delivery or maternal behavior. Therefore, the NOAEL for reproductive toxicity was 1,000 mg/kg/day.

Developme ntal toxicity

Two studies by oral administration and one study by inhalation are available. An oral study by MHW (OECD preliminary reproduction toxicity screening test) [MHW, Japan: 2002] and an inhalation study by Gaworski et al. [1992] were identified as the key study. MHW study was well conducted and reported according to OECD TG 421 and GLP although there is no sufficient evidence on teratogenicity because the prenatal examination was not scheduled. Gaworski's study was well conducted and reported, although it was not conducted under GLP and published test guidelines. The reliability of another oral study [Ana Cristina et al.: 1995] is low because there are several significant changes with no dose-dependency at the lowest dose (60 mg/kg) to 1,000 mg/kg, while MHW study and Gaworski's study showed no fetal effects up to 200 mg/kg and approx. 77 mg/kg, respectively.

In an OECD preliminary reproduction toxicity screening test, citral was administered to Crj:CD(SD) rats by gavage at doses of 0, 40, 200 and 1,000 mg/kg/day in males for 46 days including before and during mating period, and in females for 39-50 days including before and through mating and gestation periods and until day 3 of lactation [MHW, Japan: 2002].

Body weights of male and female pups were reduced in the 1000 mg/kg group. There was no effect on viability and morphogenesis of pups. The NOAEL for developmental toxicity was considered to be 200 mg/kg/day.

SD pregnant rats were exposed to citral by inhalation for 6 hr/day on gestation days 6-15 at mean concentration of 0, 10 or 34 ppm as vapour, or 68 ppm as an aerosol/vapour mixture [Gaworski et al.: 1992]. Dams were killed on gestation day 20 and the fetuses were examined.

No significant maternal toxicity or teratogenic effects were observed at concentrations up to 34 ppm. Significant maternal toxicity such as reduction of body weight, abortion (one female, on day 10 of gestation) and death (one female, on day 17 of gestation) were observed at 68 ppm. However, even in the presence of the maternal effects, no significant teratogenicity was noted at 68 ppm. A NOAEL of teratogenicity was established at 68 ppm (423 mg/m3) (equivalent to 77 mg/kg/day).

There is no available information on humans.

Conclusions:

Only reduction of body weights in pups was observed in a preliminary reproduction toxicity test. Therefore, the NOAEL of developmental toxicity by oral administration was considered to be 200 mg/kg/day. In an inhalation teratogenicity study, any teratogenicity was not observed even at the highest dose level of 68 ppm (423 mg/m3) (equivalent to 77 mg/kg/day).

e) Genotoxicity
The summary of genotoxicity of citral was given in the following Table 6.

Table 6: Summary of genotoxicity studies

Type of test	Test system	Dose	Result	Reference
BACTERIAL TEST				
Ames test *(reverse mutation)*	*S. typh.* (TA 102, TA 100, TA 98, TA 97a)	5 to 700 ug /plate	Negative (+ & - MA)	Gomes-Carneiro et al.: 1998
Ames test *(reverse mutation)*	*S. typh.* (TA100)	No data	Negative (+ & - MA)	Eder et al.: 1982
Ames test *(reverse mutation)*	*S. typh.* (TA 92, TA 94, TA 98, TA 100, TA 1535, TA 1537)	up to 100 ug /plate	Negative (+ & - MA)	Ishidate et al.: 1984
Ames test *(reverse mutation)*	*S. typh.* (TA 98, TA 100, TA 1535, TA 1537)	up to 10 mg /plate	Negative (+ & - MA)	Zeiger et al.: 1987
Ames test *(reverse mutation)*	*S. typh.* (TA100)	No data	Negative (+ & - MA)	Lutz et al.: 1982
Ames test *(reverse mutation)*	*S. typh.* (TA 98, TA 100, TA 1535, TA 1537)	up to 220 ug/plate	Negative (+ & - MA)	NTP: 2001
Recombination assay	*Bacillus subtilis M 45 (rec -), H 17 (rec +)*	No data	Positive	Yoo: 1985
Reverse mutation	*Escherichia coli WP2 uvrA (trp -)*	No data	Negative	Ishidate et al.: 1984
NON-BACTERIAL TEST IN VITRO				
Cytogenetic assay	CH cell	up to 30 ug/ml	**Negative** (- MA)	Ishidate et al.: 1983
Cytogenetic assay	CH cell	up to 70.7 ug/ml	Negative (+ & - MA)	NTP: 2001
Sister chromatid exchange assay	CH cell	up to 40.2 ug/ml	Positive (+ & - MA)	NTP: 2001
GENETIC IN VIVO TEST				
Micronucleus test (Bone marrow)	Male B6C3F1 mouse	up to 750 mg/kg/day* (i.p. for 3days)	Negative	NTP: 2001
Micronucleus test (erythrocytes)	Male and female B6C3F1 mouse	up to 31,300 ppm (capsule for 14 weeks)	Negative	NTP: 2001

MA: metabolic activation
*Since the highest dose, 1000 mg/kg was lethal, this group was not examined.

Bacterial in vitro test
Seven bacterial reverse mutation studies indicate negative results with and without metabolic activation [Eder et al.: 1982, Lutz et al.: 1982, Ishidate et al.: 1984, Yoo: 1985, Zeiger et al.: 1987, Gomes-Carneiro et al.: 1998, NTP: 2001]. Although there are no studies using published test guidelines and under GLP, the following study was well conducted and the details of data were reported.

A reverse mutation test was conducted with *Salmonella typhimurium* TA97a, TA98, TA100 and TA102 with and without metabolic activation [Gomes-Carneiro et al.: 1998]. Negative results were given up to maximum concentrations such as 700 ug/plate in TA100, TA98 and TA97a, or 300 ug/plate in TA102 without S9 and 700 ug/plate in TA98 and TA97a, 600 ug/plate in TA100 or 400 ug/plate in TA102 with S9.

Non-bacterial in vitro test
Two chromosomal aberration results in Chinese hamster cells are negative [Ishidate et al.: 1984, NTP: 2001] while one positive result in sister chromatid exchange is given in the same cells [NTP: 2001]. Ishidate's study was well conducted under a project operated by MHW, Japan and the detail is as follows.

Mammalian cultured CHL/IU cells were treated for 24 or 48 hrs for continuous treatment without S9 [Ishidate et al.: 1984]. Clastogenicity and polyploidy were not induced up to 0.03 mg/ml (maximum dose, needed for 50% cell growth inhibition).

Genetic in vivo test
Two micronucleus tests (bone marrow and erythrocyte) conducted by NTP (2001) showed negative results. In the bone marrow micronucleus test, male mice received intraperitoneal injections at doses of 250 to 750 mg/kg/day daily for 3 days. In another study, sampling was conducted from peripheral blood within 24 hours after 14-week feeding study, in which male and female B6C3F1 mice were given up to 31,300 ppm microencapsulated citral in food.

Conclusions:
Seven bacterial reverse mutation studies indicate negative results with and without metabolic activation. As for non-bacterial *in vitro* study, negative results were given in two chromosomal aberration tests but positive result in one sister chromatid exchange assay. Two *in vivo* micronucleus tests indicate negative results. Based on weight of evidence, the genotoxic potential of citral can be considered to be negative.

f) Carcinogenicity
A 2-year NTP study (2001) with citral using rats and mice was conducted. In this study, microencapsulated citral was given for 2 years to F344/N rats (50/sex/dose) at 0 (untreated), 0 (vehicle), 1,000, 2,000 or 4,000 ppm (approximately 50, 100 and 210 mg/kg, respectively) and B6C3F1 mice (50/sex/dose) at 0 (untreated), 0 (vehicle), 500, 1,000, or 2,000 ppm (approximately 60, 120, 260 mg/kg, respectively). No treatment-related neoplastic changes were observed in male/female rats and female mice. Only in female mice, the incidences of malignant lymphoma occurred with a positive trend (3/49, 5/50, 9/50, 12/50 at 0 (vehicle), 500, 1,000 and 2,000 ppm, respectively) and significantly increased at the highest dose group. Tissues most commonly affected by malignant lymphoma were the spleen, mesenteric lymph node, thymus, and to a lesser extent, the ovary.

Conclusions:
There was no evidence of carcinogenic activity of citral in male or female F344/N rats exposed to 1,000, 2,000, or 4,000 ppm. There was no evidence of carcinogenic activity of citral in male B6C3F1 mice exposed to 500, 1,000, or 2,000 ppm. But there was some evidence of carcinogenic activity in female B6C3F1 mice, based on increased incidences of malignant lymphoma.

g) Other human health related information

Irritation and sensitization

- The undiluted 0.5 ml citral caused moderate erythema and edema to the skin in rabbits [EPA/OTS: 1992].
- Citral was moderately to markedly irritating to skin in rabbits [Moreno: 1974].
- Citral was no irritating to eyes in rabbits [BASF AG: 1978].

- Citral was sensitizing in guinea pig at 1 % in vaseline (the highest non-irritating concentration) [Majeti & Suskind: 1976/1977, Suskind et al.: 1976]

Human data

In a cumulative irritation study, the 8 % concentration was found to be marginal irritant after 21-days exposure [Maibach: 1971]. On the other hands, numerous samples of citral tested at 1 to 8 % produced no irritation after 48-hr closed patch tests on twelve different panels of human subjects [Epstein: 1974, Kligman: 1971, 1972 & 1974].

During an investigation of an outbreak of dermatitis following the introduction of a lemon-scented detergent, citral was shown by patch tests to be a strong primary irritant if applied in association with heat; 10 % citral induced slight responses at 23 °C and pronounced responses at 43 °C [Rothenborg et al.: 1977].

A survey of sensitisation data from tests on materials containing citral was conducted under the auspices of the Soap and Detergent Association (SDA) [Steltenkamp et al.: 1980]. This survey was restricted to skin patch tests on human subjects conducted in the USA by member companies of SDA and by perfume suppliers. None of the personal care or household products containing citral induced hypersensitivity attributed to citral in 10,660 patch tests and there were no confirmed reactions to citral in 2,098 patch tests on fragrance blends containing the substance. A total of 22 induced sensitization occurred in 174 tests conducted at 1 to 5% pure citral in ethanol, but no inductions occurred at the 0.5% citral concentration level in 82 test subjects.

Conclusions:

Citral is irritating to skin and not irritating to eyes in rabbits, and sensitizing to skin in guinea pigs. In human, this chemical was irritating to skin marginally at 8 %. Although 22 in 174 tests indicated sensitization by 1 to 5 % pure citral in ethanol, any consumer products containing this chemical did not show the effect. Therefore, this chemical was considered not to irritate and sensitize as used in consumer products.

A weak peroxisome proliferating activity

Citral induced histopathological changes in liver of rats and mice orally treated with citral only at extremely high doses. [Jackson et al.: 1987, Dieter et al.: 1993]. In Jackson's rat study, specific biochemical marker supported the morphological changes in the peroxisomes and a peroxisome-associated polypeptide of molecular weight 80,000 daltons (PPA-80) was induced. Therefore, peroxisome proliferation is involved in the action of citral on liver.

Anti-promoting activity

The ability of citral to modulate tumor promotion was tested in a two-stage skin carcinogenesis study in hairless mice [Connor: 1991]. The dorsal skins of female skh/hr1 mice were initiated with 0.1 umol dimethylbenzanthracene, and tumors were promoted by twice-weekly application of 10 nmol of tetradecanionyl-phorbol- 13-acetate (TPA) for 20 weeks. Prior to each TPA application, citral was given with 0, 1 or 10 umol. Citral had a dose-dependent inhibitory effect on tumor production in the TPA promoted groups.

Prostate glands proliferating activity

Citral induces benign or atypical hyperplasia in ventral prostate of rats by dermal application at 100-200 mg/kg/day [Servadio et al.: 1986, Abramovici et al.: 1987, Geldof et al.: 1992, Engelstein et al.: 1996].

Typical lesions of benign prostatic hyperplasia (BPH) were induced in the ventral prostate of adolescent male rats dermally treated at 150 mg/kg/day of citral [Servadio C. et al., 1986]. The microscopic examination of the ventral prostate of the animals showed definite lesions: hyperplasia in few acini were seen after 10 days of treatment, thereafter, expanding with continued treatment. After 90 days of treatment, the enlarged acini were more crowded, and reached a back-to-back arrangement while separated by dense connective tissue, which is characteristic of glandular hyperplasia. Castration prior to citral administration prevented such BPH changes [Servadio C. et al., 1986]. On the other hands, BPH was induced in castrated rats with testosterone implants [Engelstein et al.: 1996]. Changing administration schedule (every four days at 12 am instead of a daily at 8 am) and site of the application induced atypical acinar hyperplasia of the ventral prostate, instead of BPH [Abramovici et al.: 1987]. Wistar and Sprague-Dawley rat strains were the most susceptible to developing benign and atypical prostatic hyperplasia in intact and castrated rats whereas F344 and ACl/Ztm rat strains remained refractory to all treatments Skolnik et al (1994). These authors speculated that strain genotype and, more precisely, their endocrine background, may play a determinative role in the prostatic responsiveness to citral.

Since prostate hyperplasia only occurs in some rat strains, at relatively high dermal dose, and the severity of effect is low, this finding may not be relevant to humans. Furthermore, the NTP oral carcinogenicity studies indicate neither neoplastic nor non-neoplastic lesions in all male reproductive organs including prostate of rats and mice [NTP: 2001]. Therefore, further work on this effect of citral on prostate is not considered to be necessary.

Estrogenic activity

A uterotrophic test on citral was conducted using Wistar rats. Citral FCC was orally administered three times daily at 0, 500 and 1,000 mg/kg. On the basis of the uterus weights, citral did not show an estrogen-like potential [BASF: 1999].

Prediction of carcinogenesis by computer analysis

A recent prediction for citral carcinogenicity made on the basis of computer tests (COMPACT: Computer Optimised Molecular Parametric Analysis for Chemical Toxicity) was negative [Lewis et al.: 1996].

4.2 Initial Assessment for Human Health

Citral was rapidly absorbed from the gastro-intestinal tract. Much of an applied dermal dose was lost due to its extreme volatility, but the citral remaining on the skin was fairly well absorbed. Citral was rapidly metabolized and excreted as metabolites. Urine was major route of elimination.

Acute toxicity of citral is low in rodents because the oral or dermal LD50 values were more than 1000 mg/kg. This chemical is irritating to skin and not irritating to eyes in rabbits, and sensitizing to skin in guinea pigs. In human, this chemical was irritating and sensitizing to the skin at high concentrations but clinical survey showed there is no risk from consumer products.

Several repeated dose oral studies show no adverse effect of citral at less than 1,000 mg/kg exposure and some histological changes in nasal cavity or forestomach of the first exposure sites, probably due to irritation, at more than 1,000 mg/kg. Male and female F344/N rats received microencapsulated citral in feed at concentrations of 0, 0.63, 1.25, 2.5, 5 and 10% (resultant doses: 0, 142, 285, 570, 1,140 and 2,280 mg/kg/day) for 14 days. Minimal to mild hyperplasia and/or squamous metaplasia of the respiratory epithelium was observed in nasal cavity without

inflammatory response at 1,140 and 2,280 mg/kg of both sexes. The NOAEL was established at 570 mg/kg/day. In an OECD preliminary reproduction toxicity screening test [TG 421], citral was administered to Crj:CD (SD) rats by gavage at doses of 0, 40, 200 and 1,000 mg/kg/day in males for 46 days and in females for 39-50 days including before and through mating and gestation periods and until day 3 of lactation. Squamous hyperplasia, ulcer and granulation in lamina propria were observed in the forestomach at 1,000 mg/kg of both sexes. The NOAEL for repeated dose toxicity was 200 mg/kg/day for both sexes.

As for reproductive toxicity in the above preliminary reproductive study, no effects were detected in reproductive ability, organ weights or histopathology of the reproductive organs of both sexes, and delivery or maternal behavior. However, body weights of male and female pups were reduced in the 1000 mg/kg group. Therefore, a NOAEL for developmental toxicity was 200 mg/kg/day. In a teratogenicity study, SD pregnant rats were exposed to citral by inhalation for 6 hr/day on gestation days 6-15 at mean concentration of 0, 10 or 34 ppm as vapour, or 68 ppm as an aerosol/vapour mixture. Even in the presence of the maternal effects, no significant teratogenicity was noted at 68 ppm (423 mg/m3).

Seven bacterial reverse mutation studies indicate negative results with and without metabolic activation. As for non-bacterial in vitro study, two chromosomal aberration results in Chinese hamster cells are negative however one positive result in sister chromatid exchange is given in the same cells. Additionally, two in vivo micronucleus tests in rodents indicate negative results. Based on the above information, the genotoxic potential of citral can be considered to be negative.

NTP study shows that there was no evidence of carcinogenic activity in male/female rats and male mice but some evidence of malignant lymphoma in female mice.
Dermal application of citral induces prostate hyperplasia with low severity only in some strains of rats. However, the NTP oral carcinogenicity studies in rats and mice found no evidence of lesions (neoplastic or non-neoplastic) in any male reproductive organ, including the prostate. The health significance of the effects seen in the dermal studies in rats is uncertain due to dramatic strain differences and it is noted that the work has primarily been performed in a single laboratory. Therefore, further work on this effect of citral on prostate is not considered to be necessary.

5. Conclusions and Recommendations

5.1 Conclusions

Physical/chemical property, production, use and distribution

Production volume of Citral in Japan was 1200 tonnes in 1990 - 1999. This chemical is used as a food flavor and as an intermediate for perfumery and vitamin A. This chemical is rapidly decomposed at pH 4 (half-life time: Neral: 9.54 days, Geranial: 9.81days) and at pH 9 (half-life time: Neral: 30.1 days, Geranial: 22.8 days), but slowly decomposed at pH 7 (half-life time: Neral: 230 days, Geranial: 106 days). This chemical is classified as "readily biodegradable". According to a Japanese manufacturer, 1,200 kg/year (estimated) of Citral are treated in a waste water treatment plant and then released with 5,000 t/year of effluent into a river (flow rate is 1.6 x 10^{11} t/year). Local predicted environmental concentration (PEC_{local}) is $7.5x10^{-7}$ mg/l, employing a calculation model. The highest exposure to the general population via the environment would be expected through drinking water processed from surface water. The concentration in drinking water is assumed to be less than $7.5 x10^{-7}$ mg/l. Occupational exposures at production sites may occur by the inhalation and dermal route. The estimated human exposure of a worker who operates the drum filler and does sampling without protective equipment is 0.34 mg/kg/day. However, protective measures i.e. safety glasses and gloves are used during these processes. Therefore, the actual exposure to workers is lower than the estimated value.

Environment

Citral is readily biodegradable (92%, BOD), and its bioaccumulation potential seems to be low based on Log P_{ow} (2.8-3.0). This chemical has been tested in a limited number of aquatic species. For algae (*Selenastrum capricornutum*), 72 h EC_{50} (biomass) is 5 mg/L and 72 h NOEC (biomass) is 3.1 mg/L. For *Daphnia*, acute toxicity of 10 mg/l (24 h EC_{50}, immobilization) and 7 mg (48h EC_{50}, immobilization), and chronic values of 1.0 mg/L (21 d NOEC, reproduction) have been reported. Only acute toxicity value has been reported for fish, which is 4.1 mg/L (96 h LC_{50}) for *Oryzias latipes*. A PNEC of 0.01 mg/L for the aquatic organisms was calculated from the chronic toxicity value of *Daphnia* using an assessment factor of 100.

Human health

Acute toxicity of citral is low in rodents because the oral or dermal LD50 values were more than 1000 mg/kg. This chemical is irritating to skin and not irritating to eyes in rabbits, and sensitizing to skin in guinea pigs. In humans, this chemical was irritating and sensitizing to the skin at high concentrations but not by consumer products. Several repeated dose oral studies show no adverse effect of citral at less than 1,000 mg/kg for 5 days to 13 weeks exposure and some histological changes in the nasal cavity or forestomach, the first exposure sites, probably due to irritation, at more than 1,000 mg/kg. The NOAEL for repeat dose toxicity was 200 mg/kg/day. As for reproductive/developmental toxicity, only a reduction of body weights in pups was observed noted in a preliminary reproduction toxicity test. Therefore, the NOAELs for reproductive and developmental toxicity were established at 1,000 and 200 mg/kg/day, respectively. In an inhalation teratogenicity study, no developmental toxicity was observed even at the highest dose level of 68 ppm (423 mg/m3) (equivalent to 77 mg/kg/day). Seven bacterial reverse mutation studies indicate negative results with and without metabolic activation. As for non-bacterial in vitro study, negative results were given in two chromosomal aberration results but a positive result in one sister

chromatid exchange assay. Two in vivo micronucleus tests indicate negative results. Based on weight of evidence, genotoxic potential of citral can be considered to be negative.

5.2 Recommendations

The chemical is currently of low priority for further work

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IUCLID Data Set

Existing Chemical	ID: 5392-40-5
CAS No.	5392-40-5
EINECS Name	citral
EINECS No.	226-394-6
Molecular Formula	C10H16O
Producer Related Part	
Company:	ECB - Existing Chemicals
Creation date:	23-OCT-1995
Substance Related Part	
Company:	ECB - Existing Chemicals
Creation date:	23-OCT-1995
Printing date:	09-JUN-2000
Revision date:	23-OCT-1995
Date of last Update:	**23-OCT-1995**
Chapter (profile):	Chapter: 1, 2, 3, 4, 5, 7
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC

1. GENERAL INFORMATION

1.0.0 Details on Template

1.0.1 OECD and Company Information

Name: BASF AG
Street: Karl-Bosch-Str
Town: 67056 Ludwigshafen
Country: Germany

Flag: non confidential

1.0.2 Location of Production Site

1.0.3 Identity of Recipients

1.1 General Substance Information

Substance type: organic
Physical status: liquid
Flag: non confidential

1.1.1 Spectra

1.2 Synonyms

2,6-Octadienal, 3,7-dimethyl- (8CI, 9CI)
Source: BASF AG Ludwigshafen

3,7-Dimethyl-2,6-octadienal
Source: BASF AG Ludwigshafen

Citral
Source: BASF AG Ludwigshafen

1.3 Impurities

1.4 Additives

1.5 Quantity

1.6.1 Labelling

Labelling: as in Directive 67/548/EEC
Symbols: Xi
Specific limits: no
R-Phrases: (38) Irritating to skin
(43) May cause sensitization by skin contact
S-Phrases: (2) Keep out of reach of children
(24/25) Avoid contact with skin and eyes
(37) Wear suitable gloves
Flag: non confidential

1.6.2 Classification

Classification: as in Directive 67/548/EEC
Class of danger: irritating
R-Phrases: (38) Irritating to skin
Flag: non confidential

Classification: as in Directive 67/548/EEC
Class of danger:
R-Phrases: (43) May cause sensitization by skin contact
Flag: non confidential

1.7 Use Pattern

1.7.1 Technology Production/Use

1.8 Occupational Exposure Limit Values

Type of limit: MAK (DE)
Limit value:
Remark: Kein MAK-Wert festgelegt
Source: BASF AG Ludwigshafen

(1)

1.9 Source of Exposure

1.10.1 Recommendations/Precautionary Measures

1.10.2 Emergency Measures

1.11 Packaging

1.12 Possib. of Rendering Subst. Harmless

1.13 Statements Concerning Waste

1.14.1 Water Pollution

Classified by: other: BASF
Labelled by: other: BASF
Class of danger: 1 (weakly water polluting)
Source: BASF AG Ludwigshafen

1.14.2 Major Accident Hazards

Legislation: Stoerfallverordnung (DE)
Substance listed: no
Source: BASF AG Ludwigshafen

(2)

1.14.3 Air Pollution

Classified by:
Labelled by:

Number:
Class of danger:
Remark: bisher keine Zuordnung
Source: BASF AG Ludwigshafen

1.15 Additional Remarks

1.16 Last Literature Search

1.17 Reviews

1.18 Listings e.g. Chemical Inventories

2. PHYSICO-CHEMICAL DATA

2.1 Melting Point

Value: < - 10 °C
Decomposition: Yes **[ ]** No **[X]** Ambiguous **[ ]**
Sublimation: Yes **[ ]** No **[X]** Ambiguous **[ ]**
Method:
GLP: Yes **[X]** No **[ ]** ? **[ ]**

(3)

Value: < -20 degree C
Remark: Thermische Zersetzung > 190 Grad C
Source: BASF AG Ludwigshafen

(4)

2.2 Boiling Point

Value: 226 - 228 °C
Pressure:
Decomposition: Yes **[ ]** No **[X]** Ambiguous **[ ]**
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**

(3)

Value: 225 degree C at 1013 hPa
Source: BASF AG Ludwigshafen

(4)

2.3 Density

Type: density
Value: = .88 - .9 g/cm3 at 20 degree C
Source: BASF AG Ludwigshafen

(4)

2.3.1 Granulometry

2.4 Vapour Pressure

Value: < 130 Pa
Temperature: 100 °C
Method: calculated **[]**; measured **[X]**
OECD Test Guideline 104 Static method
GLP: Yes **[X]** No **[]**? **[]**

(3)

Value: < 1 hPa at 50 degree C
Source: BASF AG Ludwigshafen

(4)

2.5 Partition Coefficient

Log Pow: 2.8 (Neral)
3.0 (Geranial)
Temperature: 25°C
Method: calculated **[ ]**; measured **[X]**
OECD Test Guideline 117
GLP: Yes **[X]** No **[]**? **[]**

(5)

log Pow: = 2.695
Method: other (calculated): Inkrementenmethode von Rekker mit Computerprogramm der Firma CompuDrug Ltd.
Year:
Source: BASF AG Ludwigshafen

(6)

log Pow: = 2.76 at 25 degree C
Method: OECD Guideline 107 "Partition Coefficient (n-octanol/water),Flask-shaking Method"
Year:
Source: BASF AG Ludwigshafen

(7)

2.6.1 Water Solubility

Value: 590 mg/L
Temperature: 25 °C
Description: Miscible **[ ]**; Of very high solubility **[ ]**; Of high solubility **[ ]**; Soluble **[ ]**; Slightly soluble **[X]**; Of low solubility **[ ]**; Of very low solubility **[ ]**; Not soluble **[ ]**
Method: OECD Test Guideline 105
GLP: Yes **[ ]** No **[X]** ? **[ ]**

(5)

Value: = .42 g/l at 25 degree C
Qualitative: slightly soluble
Remark: pH neutral
Source: BASF AG Ludwigshafen

(8) (4)

2.6.2 Surface Tension

2.7 Flash Point

Value: = 98 degree C
Type:
Method: other: DIN 51 758
Year:
Source: BASF AG Ludwigshafen

(4)

2.8 Auto Flammability

Value: = 225 degree C
Method: other: DIN 51 794
Remark: Gefahr der Selbstentzuendung, wenn infolge feiner Verteilung eine grosse Oberflaeche entsteht.
Source: BASF AG Ludwigshafen

(4)

2.9 Flammability

2.10 Explosive Properties

Result:
Remark: Explosionsgrenzen in Luft: 4,3-9,9 Vol.%
Source: BASF AG Ludwigshafen

(4)

2.11 Oxidizing Properties

2.12 Additional Remarks

Remark: Zu vermeidende Stoffe: Saeuren und Basen
Source: BASF AG Ludwigshafen

(4)

3. ENVIRONMENTAL FATE AND PATHWAYS

3.1.1 Photodegradation

Type: Air **[ ]**; Water **[X]**; Soil; Other **[ ]**
Light source: Sunlight **[X];** Xenon lamp **[ ];** Other **[ ]**
Spectrum of substance:
epsilon = 6.52 x 10^3 at 300 nm
Estimated parameter for calculation:

Quantum yield	0.01
Concentration	5 x 10^{-5} M
Depth of water body	500 cm
Conversion constant	6.023 x 10^{20}
Result: Degradation rate	9.68 x 10^{-13} mol/l/s
Half life	1.14 years

(9)

Type: Air [X]; Water [ ]; Soil [ ]; Other [ ]
Light source: Sun light [ ]; Xenon lamp [ ]; Other [ ]
Light spectrum: . . . . . nm
Relative intensity:
Spectrum of substance:
Concentration of Substance:
Temperature:
Indirect Photolysis:
Type of sensitizer: OH
Concentration of sensitizer:
Rate constant (radical):136.0296 x 10^{-12} cm3/molecule*sec
Degradation: 50% after 2.83 hours
Method: calculated [X]; measured [ ];
GLP: Yes [ ] No [X] ? [ ]
Test substance:
Remarks: The reaction rate constant with OH radical was estimated by SRC AOPWIN v1.86. The half-life (2.83 hours) was calculated based on the calculated rate constant and OH radical concentration in atmosphere of 500,000 molecules/cm^3.
Reference:

Type: Indirect photolysis
Sensitizer: O_3
Method:
Year:
GLP:
Test substance:
Remark: K=4.42E-16 cm3/mol*s; berechnet mit AOP nach Meylan
Source: BASF AG Ludwigshafen

(10)

Type: other
Method:
Year:
GLP:
Test substance:
Remark: K=1.28E-10 cm3/mol*s; berechnet mit AOP nach Meylan
Source: BASF AG Ludwigshafen

(10)

3.1.2 Stability in Water

Type: Abiotic (hydrolysis) **[X]**; biotic (sediment)**[ ]**

Result: Half life
Neral
9.54 days at pH 4
230 days at pH 7
30.1 days at pH 9
Geranial
9.81 days at pH 4
106 days at pH 7
22.8 days at pH 9
Method: OECD Test guideline 111
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance: Citral

(5)

Type:
Method: other
Year:
GLP:
Test substance:
Remark: no data available
Source: BASF AG Ludwigshafen

3.1.3 Stability in Soil

Type: other
Radiolabel:
Concentration:
Cation exch. capac.
Microbial biomass:
Method:
Year:
GLP:
Test substance:
Remark: no data available
Source: BASF AG Ludwigshafen

3.2 Monitoring Data (Environment)

Type of measurement:
Medium: biota
Remark: Citral (in solution with hydroxylamin hydrochloride) has been detected in lemon-grass essence (approx. 67-69%) bypotentiometric titration.
Source: BASF AG Ludwigshafen
Test condition: pH = 3.4

(11)

3.3.1 Transport between Environmental Compartments

Type: other
Media:
Method:
Year:
Remark: no data available
Source: BASF AG Ludwigshafen

3.3.2 Distribution

Fugacity Calculation

The potential environmental distribution of citral obtained from a generic level III fugacity model under three emission scenarios is shown in Table.

Environmental distribution of citral using a generic level III fugacity model under three emission scenarios.

Compartment	Release: 100% to air	Release: 100% to water	Release: 100% to soil
Air	97.7 %	1.7 %	0.1 %
Water	1.6 %	97.0 %	0.2 %
Soil	0.7 %	0.0 %	99.7 %
Sediment	0.0 %	1.3 %	0.0 %

Media: Air, Water, Soil and Sediment
Method: Generic level III fugacity model
Year: 2001
Remark:
Source: (12)

3.4 Mode of Degradation in Actual Use

Remark: no data available
Source: BASF AG Ludwigshafen

3.5 Biodegradation

Type: aerobic [X]; anaerobic [ ]
Inoculum: adapted [ ]; non-adapted [X];
Concentration of the chemical:
100 mg/l related to Test Substance [X]
Medium: water[ ];water-sediment[ ];soil [ ];sewage treatment [ ]
other [Japanese standard activated sludge]
Degradation: Degree of degradation after 28 days
93, 88 and 94 % from BOD
76, 76 and 82 % from TOC
100, 100 and 100 % from GC analysis
Results: Readily biodeg. [X]; Inherently biodeg. [ ]; under test condition no biodegradation observed [ ]
Method: OECD Test Guideline 301 C
GLP: Yes [X] No [ ] ? [ ]
Test substance: Citral
Reference: MITI, Japan (1992)
(3)

Type: aerobic [ ]; anaerobic [X]
Inoculum: adapted [ ]; non-adapted [ ];
Concentration of the chemical:
related to Test Substance [ ]
Medium: water[ ];water-sediment[ ];soil [ ];sewage treatment [ ];
other [ ]
Degradation: Degree of degradation after 28 days
70 %
Results: Readily biodeg. [X]; Inherently biodeg. [ ]; under test condition no biodegradation observed [ ]
Method: OECD Test Guideline 301 C

GLP: Yes [ ] No [ ] ? [X]
Test substance: Citral
Reference: BASF AG

Type: aerobic
Inoculum: other: Belebtschlamm einer komunalen Laboranlage
Concentration: 90 mg/l related to Test substance
Degradation: > 90 % after 28 day
Result: readily biodegradable
Method: OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"
Year:
GLP:
Test substance:
Remark: lag-Phase: 3 d; Beginn Plateauphase: 7 d
Source: BASF AG Ludwigshafen

(13)

Type: aerobic
Inoculum: activated sludge
Concentration: 100 mg/l related to Test substance
Degradation: ca. 88 - 94 % after 28 day
Method: other: MITI-Test (BOD of THOD)
Year:
GLP:
Test substance:
Source: BASF AG Ludwigshafen
Test condition: Concentration of sludge: 30 mg/l

(14)

3.6 BOD5, COD or BOD5/COD Ratio

Method: other: BSB-Bestimmung in 5 Tagen nach DIN 38409 Teil 51
COD:

Method: other: CSB-Bestimmung nach DIN 38409 Teil 41
COD: = 1990 mg/g substance

R A T I O B O D 5 / C O D

BOD5/COD: = .28

Remark: BOD5 =556 mg/g; COD (Direkteinwaage) =3067 mg/g
Source: BASF AG Ludwigshafen

(15)

3.7 Bioaccumulation

Species: other
Exposure period:
Concentration:
BCF:
Elimination:
Method:
Year:
GLP:
Test substance:
Remark: no data available
Source: BASF AG Ludwigshafen

3.8 Additional Remarks

Remark: Natural occurrence:
Constituent (75 to 85%) of oil of lemon grass, the volatile oil of cymbopogon citratus (DC) stapf, or of cymbopogon flexuosus (nees) stapf, gramineae. Also present to a limited extent in oils of verbena, lemon, and orange.

Source: BASF AG Ludwigshafen

(16)

Remark: Natural occurrence:
found in: Litsea citrata (approx. 90%), Litsea cubeba Blume (approx. 70%), Lindera citriodora (approx. 65%), Backhousia citriodora (approx. 95-97%), Calypranthes parriculata (approx. 62%), Leptospermum liversidgei var. A.leaves (approx. 70-80%), and Ocimum gratissimum (approx. 66.5%). Also present in lemon (2-5%), lime (6-9%), and Citrus aurantifolia leaves (petitgrain, approx. 36%).

Source: BASF AG Ludwigshafen

(17)

4. ECOTOXICITY

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
NOEC: 4.6
LC0: 4.6
LC50: 4.6 - 10
LC100: 10
Method: other: nach DIN 38 412, Testverfahren mit Wasserorganismen Gruppe L, Teil L15
Year: 1982 **GLP:** no
Test substance: as prescribed by 1.1 - 1.4
Source: BASF AG Ludwigshafen

(18)

Type: semistatic
Species: Oryzias latipes (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC50: = 4.1
Method: OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year: **GLP:** no
Test substance:
Remark: A group of 10 fish were exposed to each of 5 nominal concentrations (1.0-10 mg/l). Stock solution was prepared with DMSO (550 mg/l). Controls with and without this vehicle were taken for test.
Test substance: Wako Pure Chemical Industries - purity = 98.9 % (Lot. TWQ2420), no information on isomer ratio

(19)

4.2 Acute Toxicity to Aquatic Invertebrates

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:**
EC0: = 6.25
EC50: = 11
EC100: = 25
Method: Directive 84/449/EEC, C.2 "Acute toxicity for Daphnia"
Year: **GLP:**
Test substance:
Remark: Geprueft mit Tween 80 als Loesungsvermittler.
Source: BASF AG Ludwigshafen

(20)

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:**
EC0: = 3.13
EC50: = 7
EC100: = 25
Method: Directive 84/449/EEC, C.2 "Acute toxicity for Daphnia"
Year: **GLP:**

Test substance:
Remark: Geprueft mit Tween 80 als Loesungsvermittler.
Source: BASF AG Ludwigshafen

(20)

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC50: = 10
Method: OECD Guide-line 202, part 1 "Daphnia sp., Acute Immobilisation Test"
Year: **GLP:** no
Test substance:
Remark: 20 daphnids (4 replicates; 5 organisms per replicate) were exposed to each of 5 nominal concentrations (1.8-18 mg/l). Stock solution was prepared with DMSO: HCO-40=9:1(1.8-18 mg/l). Controls with and without this vehicle were taken for test.
Test substance: Wako Pure Chemical Industries - purity = 98.9 % (Lot. TWQ2420), no information on isomer ratio

(21)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Scenedesmus subspicatus (Algae)
Endpoint:
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:**
EC10: = 4.9
EC50: = 16
Method: other: Scenedesmus-Zellvermehrungs-Hemmtest, DIN 38412 Teil 9, Bestimmung der Hemmwirkung von Wasserinhaltsstoffen auf Gruenalgen
Year: **GLP:**
Test substance:
Remark: Geprueft mit Cremophor RH 40 als Loesungsvermittler.
Source: BASF AG Ludwigshafen

(20)

Species: Scenedesmus subspicatus (Algae)
Endpoint:
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
EC10: = 1.9
EC50: = 19
Method: other: Scenedesmus-Zellvermehrungs-Hemmtest, DIN 38412 Teil 9, Bestimmung der Hemmwirkung von Wasserinhaltsstoffen auf Gruenalgen
Year: **GLP:**
Test substance:
Remark: Geprueft mit Cremophor RH 40 als Loesungsvermittler.
Source: BASF AG Ludwigshafen

(20)

Species: Selenastrum capricornutum (Algae)
Endpoint: biomass
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no
NOEC: = 3.1
EC50: = 5
Method: OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year: **GLP:** no
Test substance:

Remark: The EC50 values for biomass were calculated based on 5 nominal concentrations (1.0-10 mg/l). Stock solution was prepared with DMSO (550 mg/l). Controls with and without this vehicle were taken for test.
Test substance: Wako Pure Chemical Industries - purity = 98.9 % (Lot. TWQ2420), no information on isomer ratio

(22)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: aquatic
Species: Pseudomonas putida (Bacteria)
Exposure period: 30 minute(s)
Unit: mg/l **Analytical monitoring:**
EC10: = 800
EC50: = 2100
EC90 : > 10000
Method: other: Pseudomonas-Atmungs-Hemmtest, DIN 38412 Teil 27, in Vorber., Bestimmung der Hemmwirkung von Abwasser auf die Sauerstoffzehrung von Pseudomonas putida
Year: **GLP:**
Test substance:
Remark: Die Stammloesung wurde mit Tween 80 angesetzt.
Source: BASF AG Ludwigshafen

(20)

Type: aquatic
Species: other bacteria: Belebtschlamm, adaptiert
Exposure period: 30 minute(s)
Unit: mg/l **Analytical monitoring:**
EC20 : = 100
Method: other: Test for Inhibition of Oxygen Consumption by Activated Sludge, ISO 8192
Year: **GLP:**
Test substance:
Remark: Atmungshemmung von adaptiertem Belebtschlamm. Bei sachgemaesser Einleitung in adaptierte biologische Klaeranlagen sind keine Stoerungen der Abbauaktivitaet des Belebtschlamms zu erwarten.
Source: BASF AG Ludwigshafen

(23)

Type: aquatic
Species: other bacteria: kommunaler Belebtschlamm
Exposure period: 30 minute(s)
Unit: mg/l **Analytical monitoring:**
EC50: ca. 200
EC80 : ca. 1000
EC20 : ca. 90
Method: other: Kurzzeitatmungstest
Year: **GLP:**
Test substance:
Remark: Kurzzeitatmungstest mit Direkteinwaage der Pruefsubstanz
Source: BASF AG Ludwigshafen

(24)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

4.5.2 Chronic Toxicity to Aquatic Invertebrates

Species: Daphnia magna (Crustacea)
Endpoint: reproduction rate
Exposure period: 21 day
Unit: mg/l **Analytical monitoring:** no
NOEC: = 1
EC50: = 1.6
Method: OECD Guide-line 202, part 2 "Daphnia sp., Reproduction Test"
Year: **GLP:** no
Test substance:
Remark: 40 daphnids (4 replicates; 10 organisms per replicate) were exposed to each of 5 nominal concentrations (1.0-10 mg/l). Stock solution was prepared with DMSO: HCO-40=9:1(1.0-10 mg/l). Controls with and without this vehicle were taken for test.
Test substance: Wako Pure Chemical Industries - purity = 98.9 % (Lot. TWQ2420), no information on isomer ratio

(21)

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Soil Dwelling Organisms

Type: other
Species:
Endpoint:
Exposure period:
Unit:
Method:
Year: **GLP:**
Test substance:
Remark: no data available
Source: BASF AG Ludwigshafen

4.6.2 Toxicity to Terrestrial Plants

Species:
Endpoint:
Expos. period:
Unit:
Method: other
Year: **GLP:**
Test substance:
Remark: no data available
Source: BASF AG Ludwigshafen

4.6.3 Toxicity to other Non-Mamm. Terrestrial Species

Species: other
Endpoint:
Expos. period:
Unit:
Method:
Year: **GLP:**
Test substance:
Remark: no data available
Source: BASF AG Ludwigshafen

4.7 Biological Effects Monitoring

Remark: no data available
Source: BASF AG Ludwigshafen

4.8 Biotransformation and Kinetics

Type: other
Remark: no data available
Source: BASF AG Ludwigshafen

4.9 Additional Remarks

5. TOXICITY

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

(1)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: ca. 6800 mg/kg bw
Method: other: BASF-Test
Year: **GLP:** no
Test substance: purity: unknown
Remark: Die Testsubstanz wurde als 0.681- 50%ige Emulsion in 0.5% iger waessriger Carboxymethylcellulose-Zubereitung appliziert.
Source: BASF AG Ludwigshafen

• (25)

(2)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: ca. 4950 mg/kg bw
Method: other: BASF-Test
Year: **GLP:** no
Test substance: purity: unknown
Remark: Die Testsubstanz wurde als Suspension in Gummi arabicum appliziert. Die Originalangabe lautet: LD50 ca. 5500 ul/kg.
Source: BASF AG Ludwigshafen

(26)

(3)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: = 4960 mg/kg bw
Method: other: no data
Year: **GLP:** no
Test substance: purity: unknown

(27)

(4)

Type: LD50
Species: mouse
Sex:
Number of Animals:

Vehicle:
Value: ca. 1440 mg/kg bw
Method: other: BASF-Test
Year: **GLP:** no
Test substance: purity: unknown
Remark: Die Testsubstanz wurde als Suspension in Gummi arabicum appliziert. Die Originalangabe lautet: LD50 ca. 1600 ul/kg.
Source: BASF AG Ludwigshafen

(26)

(5)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Value: = 6000 mg/kg bw
Method: other: no data
Year: **GLP:** no
Test substance: purity: unknown

(28)

(6)

Type: LD0
Species: mouse
Sex:
Number of Animals:
Vehicle:
Value: = 900 mg/kg bw
Method: other: no data
Year: **GLP:** no
Test substance: purity: unknown

(29)

5.1.2 Acute Inhalation Toxicity

(1)

Type: other: IRT
Species: rat
Sex:
Number of Animals:
Vehicle:
Exposure time: 7 hour(s)
Value:
Method: other: in Anlehnung an Smyth, H.F. et al.: Am. Ind. Hyg. Ass. J. 23, 95-107
Year: 1962 **GLP:** no
Test substance: purity: unknown
Remark: Keine Mortalitaet nach 7 Stunden Exposition in einer bei 20 Grad C angereicherten bzw. gesaettigten Atmosphaere.
Source: BASF AG Ludwigshafen

(25)

5.1.3 Acute Dermal Toxicity

(1)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: > 2000 mg/kg bw
Method: other: BASF-Test
Year: **GLP:** no
Test substance: purity: unknown
Source: BASF AG Ludwigshafen

(25)

(2)

Type: LD50
Species: rabbit
Sex:
Number of Animals:
Vehicle:
Value: = 2250 mg/kg bw
Method: other: no data
Year: **GLP:** no
Test substance: purity: unknown
Source: BASF AG Ludwigshafen

(30)

5.1.4 Acute Toxicity, other Routes

(1)

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.p.
Value: ca. 450 mg/kg bw
Method: other: BASF-Test
Year: **GLP:** no
Test substance: purity: unknown
Remark: Die Testsubstanz wurde als Suspension in Gummi arabicum appliziert. Die Originalangabe lautet: LD50 ca. 500 ul/kg.
Source: BASF AG Ludwigshafen

(26)

(2)

Type: LD50
Species: mouse
Sex:
Number of
Animals:
Vehicle:
Route of admin.: i.p.
Value: 140 - 210 mg/kg bw
Method: other: BASF-Test

Year: **GLP:** no
Test substance: purity: unknown
Remark: Die Testsubstanz wurde als 0.681 - 50%ige Emulsion in 0.5%iger waessriger Carboxymethylcellulose-Zubereitung appliziert.
Source: BASF AG Ludwigshafen

(25)

(3)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.p.
Value: ca. 360 mg/kg bw
Method: other: BASF-Test
Year: **GLP:** no
Test substance: purity: unknown
Remark: Die Testsubstanz wurde als Suspension in Gummi arabicum appliziert. Die Originalangabe lautet: LD50 ca. 400 ul/kg.
Source: BASF AG Ludwigshafen

(26)

(4)

Type: LD50
Species: mouse
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.p.
Value: = 460 mg/kg bw
Method: other
Year: **GLP:** no data
Test substance: purity: unknown
Source: BASF AG Ludwigshafen

(31)(32)

(5)
Type: LD0
Species: mouse
Sex:
Number of Animals:
Vehicle:
Route of admin.: i.p.
Value: = 250 mg/kg bw
Method: other: no data
Year: **GLP:** no
Test substance: purity: unknown
Source: BASF AG Ludwigshafen

(29)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

(1)

Species: rabbit
Concentration:
Exposure Time: 24 hours
Number of
Animals: unknown
Result: moderately to markedly irritating
EC classificat.: unknown
Method: unknown
Year: unknown **GLP:** no data
Test substance: unknown
Remark: Citral applied full strength to intact or abraded rabbit skin for 24 hr under occlusion.

(33)

(2)

Species: rabbit (New Zealand albino)
Concentration: 0.5 to 6 ml
Exposure:
Exposure Time: 24 hours
Number of
Animals: unknown
PDII: unknown
Result: corrosive
EC classificat.: unknown
Method: unknown
Year: 1992 **GLP:** no data
Test substance: unknown
Remark: The undiluted substance was applied.
Clinical signs included moderate erythema and edema, and loosening of the scab edges in 14-17 days.
Source: BASF AG Ludwigshafen

(34)

(3)

Species: rabbit
Concentration:
Exposure:
Exposure Time:
Number of
Animals:
PDII:
Result: irritating
EC classificat.:
Method: other: Aetz-Test, 1 Stunde
Year: **GLP:** no
Test substance: purity: unknown
Source: BASF AG Ludwigshafen

(25)

(4)

Species: rabbit
Concentration:
Exposure:
Exposure Time:
Number of

Animals:
PDII:
Result: irritating
EC classificat.:
Method: other: 24 Stunden, okklusiv
Year: **GLP:** no
Test substance: purity: unknown
Remark: intakte und skarifizierte Haut
Source: BASF AG Ludwigshafen

(32)

(5)

Species: rabbit
Concentration:
Exposure:
Exposure Time:
Number of
Animals:
PDII:
Result: irritating
EC classificat.:
Method: other: BASF-Test
Year: **GLP:** no
Test substance: purity: unknown
Source: BASF AG Ludwigshafen

(25)

(6)

Species: rabbit
Concentration:
Exposure:
Exposure Time:
Number of
Animals:
PDII:
Result: irritating
EC classificat.:
Method: other: Aetz-Test, 4 Stunden
Year: **GLP:** no
Test substance: purity: unknown
Source: BASF AG Ludwigshafen

(25)

(7)

Species: human
Concentration: 1, 4, 8 % in petrolatum
Exposure Time: 21 days
Number of
Animals: 8
Result: slightly irritating
EC classificat.:
Method: unknown
Year: **GLP:** no
Test substance: purity: unknown
Remarks: A cumulative irritation study was carried out on 8 volunteers. Patches were placed on the back daily, removed at 24 hr and read and them replaced with a fresh patch, over a period of 21 days. The 8 % concentration was found to be a marginal irritant.

(35)

(8)

Species: human
Concentration: 1 to 8 %
Exposure Time: 48 hours
Number of Animals:
Result: non irritating
EC classificat.:
Method: unknown
Year: **GLP:** no
Test substance: purity: unknown
Remarks: Numerous samples of citral tested at various concentrations from 1 to 8 % produced no irritation after 48-hr closed patch tests on twelve different panels of human subjects.
(36) (37)

5.2.2 Eye Irritation

(1)

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: not irritating
EC classificat.:
Method: other: BASF-Test
Year: **GLP:** no
Test substance: purity: unknown
Source: BASF AG Ludwigshafen
(25)

5.3 Sensitization

(1)

Type: Guinea pig maximization test
Species: guinea pig
Number of Animals:
Vehicle:
Result: sensitizing
Classification:
Method: other: nach Magnusson und Kligman, Allergic contact dermatitis in the guinea pig, Ch.C. Thomas, Springfield, Illinois, USA
Year: 1970 **GLP:** no
Test substance: purity: unknown
Source: Citral synthetisch
(38)

(2)

Type: Buehler Test
Species: guinea pig
Number of Animals:
Vehicle:

Result: sensitizing
Classification:
Method: other
Year: **GLP:** no
Test substance: purity: unknown
Remarks: Sensitization was induced by 1 % citral in vaseline; the highest non-irritating concentration of citral for guinea-pig skin was found to be 1 % in vaseline or 2 % in acetone.
Source: BASF AG Ludwigshafen

(39)(40)

(3)

Type: Open epicutaneous test
Species: guinea pig
Number of Animals:
Vehicle:
Result: sensitizing
Classification:
Method: other
Year: **GLP:** no data
Test substance: purity: unknown
Remark: Die Autoren diskutieren einen moeglichen "Quench"-Effekt durch gleichzeitige Applikation von Limonen.
Source: BASF AG Ludwigshafen

(41)

(4)

Type: Guinea pig maximization test
Species: guinea pig
Number of Animals:
Vehicle:
Result: sensitizing
Classification:
Method: other: nach Magnusson und Kligman, Allergic contact dermatitis in the guinea pig, Ch.C. Thomas, Springfield, Illinois, USA
Year: 1970 **GLP:** no
Test substance: purity: unknown
Source: Citral natuerlich

(42)

(5)

Type: Patch-Test
Species: guinea pig
Number of Animals: 5
Vehicle:
Result: sensitizing
Classification:
Method: other: Maguire Method
Year: **GLP:** no
Test substance: purity: unknown
Remarks: Sensitization was induced in four of five animals at 8 % citral.
Source: BASF AG Ludwigshafen

(43)

(6)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result: sensitizing
Classification:
Method: other
Year: **GLP:** no data
Test substance: purity: unknown
Remark: Nach Angabe der Autoren wirkt reines Citral in Loesungen von1 % und hoeher sensibilisierend.
Source: BASF AG Ludwigshafen

(44)

(7)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result: ambiguous
Classification:
Method: other
Year: **GLP:** no data
Test substance: purity: unknown
Remark: Nach Angabe der Autoren erzeugt Citral in Mischung (Verbraucherprodukte, Duftstoffe) keine Sensibilisierung.
Source: BASF AG Ludwigshafen

(44)

(8)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result: sensitizing
Classification:
Method: other
Year: **GLP:** no data
Test substance: purity: unknown
Remark: Fall einer Patientin mit Kontaktdermatitis, die auf Citral positiv reagierte.
Getestete Substanz: "Citral" (lt. Formel aber 2,6-Octadienal, 7-Methyl)
Source: BASF AG Ludwigshafen

(45)

(9)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result: ambiguous
Classification:
Method: other: no data

Year: **GLP:** no
Test substance: purity: unknown
Remark: According to the repeated-insult patch procedure, six reations were produced in 56 human subjects with 8 % and eleven 24-hr exposures. This was confirmed by a "use test" in which 1 % citral in petrolatum was applied to the cheeks and forearm of five subjects, two of whom developed a dermatitis at the test site.
Source: BASF AG Ludwigshafen

(35) (46)

(10)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result: ambiguous
Classification:
Method: other: no data
Year: **GLP:** no
Test substance: purity: unknown
Remark: Citral was tested by the repeated-insult patch procedure. A concentration of 8 % citral, in a test involving eleven 48-hr exposures on 105 human subjects, produced a number of reactions by the third exposure and the conentration had to be reduced to 4 %, at which level no sensitization reactions were produced.
Source: BASF AG Ludwigshafen

(47)

(11)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result: ambiguous
Classification:
Method: other: no data
Year: **GLP:** no
Test substance: purity: unknown
Remark: Citral was tested by the repeated-insult patch procedure. With 8 % (which had to be reduced to 4 % because of irritation) and eleven 24-hr exposures, 48 % of a panel of 40 human subjects were sensitized. A rechallenge patch test was then carried out on 36 of the original 40 panelists and produced six reactions in subjects who had previously reacted and two additional reactions in those who had shown little or no reaction in the original study.
Source: BASF AG Ludwigshafen

(46) (48)

(12)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result: ambiguous
Classification:

Method: other: no data
Year: **GLP:** no
Test substance: purity: unknown
Remark: Citral was tested by the repeated-insult patch procedure. Two different samples of citral, tested at 4 % using eleven 24-hr exposures, produced in the one case, five sensitization reactions in ten subjects and in the other, no reactions in a group of 50.
Source: BASF AG Ludwigshafen

(46) (49) (50)

(13)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result: sensitizing
Classification:
Method: other: no data
Year: **GLP:** no data
Test substance: purity: unknown
Remark: 2,3 % (16/680 untersuchten Patienten) reagierten positiv. Quelle ist in japanischer Sprache mit engl. Abstract und teilweise eng. Tabellen verfasst. Die Methodikdarstellung ist deswegen nicht beurteilbar.
Source: BASF AG Ludwigshafen

(51)

(14)

Type: other: maximization test
Species: human
Number of Animals:
Vehicle:
Result: sensitizing
Classification:
Method: other: nach Kligman, A.M. und Epstein, W.: Contact Dermatitis 1, 231
Year: 1975 **GLP:** no
Test substance: purity: unknown
Remark: Die Autoren gehen davon aus, dass Citral isoliert angewendetsensibilisierend wirkt, in Mischung mit anderen Pflanzeninhaltsstoffen jedoch keine sensibilisierende Wirkung entwickelt.
Source: BASF AG Ludwigshafen

(52)

(15)

Type: other: maximization test
Species: human
Number of Animals:
Vehicle:
Result: sensitizing
Classification:
Method: other: no data
Year: **GLP:** no
Test substance: purity: unknown
Remark: Getestet wurden Konzentrationen von 0.1 %- bis 2 %igen

Loesungen.
Source: BASF AG Ludwigshafen

(53)

(16)

Type: other: maximization test
Species: human
Number of Animals:
Vehicle:
Result: sensitizing
Classification:
Method: other: no data
Year: **GLP:** no
Test substance: purity: unknown
Remark: Maximization tests on human volunteers (in panels averaging 25 subjects each) were used to test various samples of citral from different sources and made by different manufacturing methods. The samples included citral ex lemongrass, citral synthetic, citral natural, citral refined and citral pure, and the following results were obtained; citral samples no. 253169 at 8 %, no. 71-3 at 4 %, no. 71-2 at 4 % and no. 71-1 at 4 % produced eight, nine, four and five sensitization reactions, respectively, per test group, sample no. DL-06R, no. DL-10R and no. 72-72 all at 4 %, produced three, five and three sensitization reactions, respectively, samples no. 23-45A, no. 23-45B, no. 23-450 and no. 23-45N, all at 5 % produced 16, 14, 8 and 12 sensitization reactions, sample no. 23-45D at 5 % produced ten seisitization reactions and sample no. 25-8-3169 at 1, 4 and 8 % produced six sensitizaion reactions.
Source: BASF AG Ludwigshafen

(35) (36) (37)

(17)

Type: patch test
Species: human
Number of **Animals:**
Vehicle:
Result: no irritation or sensitizing
Classification:
Method: other: no data
Year: **GLP:** no data
Test substance: purity: unknown
Remark: Citral was applied at a dosage of 0.5 ml to the occluded upper arms of 42 human subjects.
Source: BASF AG Ludwigshafen

(54)

(18)

Type: maximization tests
Species: human
Number of **Animals:**
Vehicle:
Result: sensitizing
Classification:
Method: other: no data
Year: **GLP:** no data
Test substance: purity: unknown

Remark: At 2 % citral produced two sensitization reactions in 24 subjects after five exposures, at 0.5 % two sensitization reactions were produced in 25 new volunteers after ten exposures, and at 0.1 % one sensitization reaction was produced in 25 new volunteers after 15 exposures. Another course of 5-48-hr exposures given to these same subjects produced two additional sensitization reactions after the 20 exposures.
Source: BASF AG Ludwigshafen

(55)

5.4 Repeated Dose Toxicity

(1)

Species: rat **Sex:** male/female
Strain: SD (Crj(CD))
Route of admin.: gavage
Exposure period: Male: 46 days, Female: 39-50 days from before mating to day 3 of lactation,throughout gestation period
Frequency of treatment: once daily
Post. obs. period: no
Doses: 40, 200, 1000 mg/kg
Control Group: yes, concurrent no treatment (corn oil)
NOAEL: 200 mg/kg for both sexes
Method: OECD Preliminary Reproduction Toxicity Screening Test (TG 421)
Year: 1997 **GLP:** yes
Test substance: purity, 98.20%
Result: Both sexes of the 1000 mg/kg group showed decrease in body weight and food consumption. Autopsy revealed partial thickening of mucosal layer in the stomach in females of the 1000 mg/kg group, and histological examination in the forestomach revealed squmous hyperplasia, ulcer and granulation in lamina propria in both sexes of the 1000 mg/kg group.

(56)

(2)

Species: rat **Sex:** male/female
Strain: Osborne-Mendel
Route of admin.: oral feed
Exposure period: 13 Wochen
Frequency of treatment: kontinuierlich im Futter
Post. obs. period: keine
Doses: 1000; 2500; 10000 ppm (entspricht ca. 83; 208; 833 mg/kg)
Control Group: yes, concurrent no treatment
NOAEL: ca. 833 mg/kg
Method: other
Year: **GLP:** no
Test substance: purity: unknown
Result: Es waren keine Auswirkungen auf die KGW-Zunahme oder haematologische Parameter zu beobachten. Morphologische Veraenderungen (Histologie nur in der hoechsten Dosierung) traten nicht auf. Die Quelle enthaelt die Informationen, dass der Substanzverlust im Futter ueber 7 Tage ca. 58 % betraegt.
Source: BASF AG Ludwigshafen

(57)

(3)

Species: rat **Sex:** male/female
Strain: Fischer 344
Route of admin.: oral feed
Exposure period: 14 days
Frequency of treatment: daily
Post. obs. period: none
Doses: 0.63; 1.25; 2.5; 5; 10 % in feed (resultant doses: 142; 285; 570; 1140; 2280 mg/kg)
Control Group: yes
NOAEL: 570 mg/kg
Method: other
Year: **GLP:** no data
Test substance: mixture of two geometric isomers (approx, 70% geranial and approx. 30 % neral)
Result: Histopathological examination revealed the changes the minimal to mild hyperplasia and /or squamous metaplasia of the respiratory epithelium of the nose without inflammatory response occurred in both sexes at 1140 and 2280 mg/kg.
Source: BASF AG Ludwigshafen

(58)

(4)

Species: rat **Sex:** male/female
Strain: Fischer 344
Route of admin.: by gavage
Exposure period: 12 days (daily for 12 days not including weekends (2 days) and 2 consecutive days of dosing before autopsy at day 14)
Frequency of treatment: daily
Post. obs. period: none
Doses: 0, 570, 1140, 2280 mg/kg
Control Group: yes
NOAEL: 1140 mg/kg for males and 2280 mg/kg for females
Method: other
Year: **GLP:** no data
Test substance: mixture of two geometric isomers (approx, 70% geranial and approx. 30% neral)
Result: Histopathological examination revealed the minimal hyperplasia of the squamous epithelium of the forestomach in males at 2280 mg/kg. No other changes related to the administration of citral by gavage were observed.
Source: BASF AG Ludwigshafen

(58)

(5)

Species: mouse **Sex:** male/female
Strain: $B6C3F_1$
Route of admin.: oral feed
Exposure period: 14 days
Frequency of treatment: daily
Post. obs. period: none
Doses: 0.63; 1.25; 2.5; 5; 10 % in feed (resultant doses: 534, 1068, 2137, 4275, 8550 mg/kg)

Control Group: yes
NOAEL: 8550 mg/kg
Method: other
Year: **GLP:** no data
Test substance: mixture of two geometric isomers (approx, 70% geranial and approx. 30% neral)
Result: No treatment-related clinical signs and pathological changes were observed.
Source: BASF AG Ludwigshafen

(58)

(6)

Species: mouse **Sex:** male/female
Strain: $B6C3F_1$
Route of admin.: by gavage
Exposure period: 12 days (daily for 12 days not including weekends (2 days) and 2 consecutive days of dosing before autopsy at day 14)
Frequency of treatment: daily
Post. obs. period: none
Doses: 0, 534, 1068, 2137mg/kg
Control Group: yes
NOAEL: 534 mg/kg
Method: other
Year: **GLP:** no data
Test substance: mixture of two geometric isomers (approx, 70% geranial and approx. 30% neral)
Result: Citral caused the death in males at 1068 mg/kg and more, and in females at 2137 mg/kg. Liver weights were increased with increasing doses in both sexes. Histopathological examination revealed that cytoplasmic vacuolisation of hepatocytes in males at 2137 mg/kg and in females at 1068 mg/kg and more, and necrosis, ulceration and inflammation of the forestomach in both sexes at 2137 mg/kg and hyperplasia and/or inflammation in both sexes at 1068 mg/kg were present.
Source: BASF AG Ludwigshafen

(58)

(7)

Species: mouse **Sex:** no data
Strain: no data
Route of admin.: i.p.
Exposure period: 5 Applikationen
Frequency of treatment: keine Angaben
Post. obs. period: 16 Tage
Doses: 90, 180, 360 mg/kg (100, 200, 400 ul/kg)
Control Group: no
Method: other: BASF-Test
Year: **GLP:** no
Test substance: purity: unknown
Result: 5 x 90 mg/kg in Gummi arab.: keine Mortalitaet (0/5)
5 x 180 mg/kg in Gummi arab.: keine Mortalitaet (0/5)
5 x 180 mg/kg in Propylenglykol: letal bei 1/5
5 x 360 mg/kg in Propylenglykol: letal bei 5/5
Source: BASF AG Ludwigshafen

(26)

5.5 Genetic Toxicity 'in Vitro'

(1)

Type: Ames test
System of testing: Salmonella typhimurium TA 102, TA 100, TA 98, TA 97a
Concentration: -S9: 300, 400, 500, 600, 700 ug/plate for TA100
200, 300, 400, 500, 600, 700 ug/plate for TA98 and 97a
5, 10, 25, 50, 100, 200, 300 ug/plate for TA102
+S9: 100, 200, 300, 400, 500, 600 ug/plate for TA100
300, 400, 500, 600, 700 ug/plate for TA98
200, 300, 400, 500, 600 700 ug/plate for TA97a
5, 10, 25, 50, 100, 200, 300, 400 ug/plate for TA102
Metabolic **activation:** with and without
Result: negative
Method: D.M. Marson, B.N.Ames, Revised methods for Salmonella mutagenicity test, Mutat. Res., 113, 173-215(1983)
Year: 1997 **GLP:** no data
Test substance: a mixture of isomers, neral and geranial, purity: unknown
Remark: S9 from rat liver, induced with Aroclor1254
Source: BASF AG Ludwigshafen

(59)

(2)

Type: Ames test
System of testing: Salmonella typhimurium TA 98, TA100, TA1535, TA1537
Concentration: 1.0, 3.3, 10.0, 33.0, 50.0, 67.0, 100.0, 160.0, 220.0 ug/plate
Metabolic activation: with and without
Result: negative
Method: other
Year: **GLP:** no data
Test substance: purity: unknown
Remark: S9 from male Sprague-Dawley rat or Syrian hamster liver, induced with Aroclor 1254
The positive controls without S9 were sodium azide (TA 100 and TA 1535), 9-aminoacridine (TA 1537), and 4-nitro-o-phenylenediamine (TA98). The positive control with S9 was 2-aminoanthracene (all strains).

(60)

(3)

Type: Ames test
System of testing: Salmonella typhimurium TA 100
Concentration: keine Angaben
Metabolic activation: with and without
Result: negative
Method: other: nach Ames, B.N. et al.: Mutation Research 31, 347
Year: 1975 **GLP:** no data
Test substance: purity: unknown
Remark: S-9
Source: BASF AG Ludwigshafen

(61)

(4)

Type: Ames test
System of testing: Salmonella typhimurium TA 92, TA 94, TA 98, TA 100, TA 1535, TA 1537
Concentration: bis zu 100 ug/Platte
Metabolic activation: with and without
Result: negative
Method: other: nach Ames, B.N. et al.: Mutation Research 31, 347
Year: 1975 **GLP:** no data
Test substance: purity: unknown
Remark: S-9
Source: BASF AG Ludwigshafen

(62)

(5)

Type: Ames test
System of testing: Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537
Concentration: bis zu 10 mg/Platte
Metabolic activation: with and without
Result: negative
Method: other: nach Haworth, S. et al.: Environ. Mutagen. 5, Suppl. 1, 3-142
Year: 1983 **GLP:** no data
Test substance: purity: unknown
Remark: S-9
Source: BASF AG Ludwigshafen

(63)

(6)

Type: Ames test
System of testing: Salmonella typhimurium TA 100
Concentration: keine Angaben
Metabolic activation: with and without
Result: negative
Method: other: nach Rannung, U. et al.: Chem.-Biol. Interact. 12, 251-263
Year: 1976 **GLP:** no data
Test substance: purity: Unknown
Remark: Citral was devoid of any mutasgenic activity both with and without S9 mix.
Source: BASF AG Ludwigshafen

(64)

(7)

Type: Bacillus subtilis recombination assay
System of testing: Bacillus subtilis M 45 (rec -), H 17 (rec +)
Concentration: keine Angaben
Metabolic activation: no data
Result: positive
Method: other: no data
Year: **GLP:** no data
Test substance: purity: unknown
Remark: Ergebnis entstammt einer Zusammenfassung.

Source: BASF AG Ludwigshafen

(65)

(8)

Type: Escherichia coli reverse mutation assay
System of testing: Escherichia coli WP2 uvrA (trp -)
Concentration: keine Angaben
Metabolic activation: no data
Result: negative
Method: other: no data
Year: **GLP:** no data
Test substance: purity: unknown
Remark: Ergebnis entstammt einer Zusammenfassung.
Source: BASF AG Ludwigshafen

(65)

(9)

Type: Cytogenetic assay
System of testing: Chinese Hamster Fibroblastenzellinie (CHL)
Concentration: bis zu 30 ug/ml
Metabolic activation: without
Result: negative
Method: other: nach Ishidate Jr., M. und Odashima, S.: Mutation Research 48, 337
Year: 1977 **GLP:** no data
Test substance: purity: unknown
Remark: Es wurden chromosomale Aberrationen untersucht
Source: BASF AG Ludwigshafen

(66)

(10)

Type: Cytogenetic assay
System of testing: Chinese Hamster Ovary (CHO) cell
Concentration: -S9: 12.5, 20.2, 25.3, 40.3 ug/ml
+S9: 30.3, 40.3, 50.5, 60.6, 70.7 ug/ml
Metabolic activation: with and without
Result: negative
Method: other:
Year: **GLP:** no data
Test substance: purity: unknown
Remark: CHO cells were treated with citral for about 10 or 20 hrs for continuous treatment with and without S9.
Positive control: Mitomycin-C up to 0.50 ug/ml (-S9)
Cyclophosphamide up to 62.5 ug/ml (+S9)

(60)

(11)

Type: Sister chromatid exchange assay
System of testing: Chinese Hamster Ovary (CHO) cell
Concentration: -S9: 0.289, 0.868, 2.890, 8.860 ug/ml (Trial 1)
2.5, 5.0, 7.5, 10.0, 20.0 ug/ml (Trial 2)
+S9: 0.87, 2.89, 8.68, 28.90 ug/ml (Trial 1)

15.1, 20.1, 25.2, 30.2, 40.2 ug/ml (Trial 2)

Metabolic activation: with and without
Result: positive
Method: other: no data
Year: **GLP:** no data
Test substance: purity: unknown
Remark: CHO cells were incubated with citral for 2 hrs with S9 and for 26 hrs without S9. All incubation time was 28 hrs both with and without S9.
Positive control: Mitomycin-C up to 10.0 ug/ml (-S9)
Cyclophosphamide up to 2.0 ug/ml (+S9)

(60)

5.6 Genetic Toxicity 'in Vivo'

(1)

Type: Micronucleus assay (mouse bone marrow)
Species: Mouse
Sex: male
Strain: B6C3F1
Route of admin.: i.p.
Exposure period: 3 days
Doses: 250, 500, 750, 1,000 mg/kg
Result: negative
Method: other: no data
Year: **GLP:** no data
Test substance: purity: unknown
Remark: Poluchromatic erythrocytes from bone marrow were scored for the frequency of micronucleated cells.
The highest dose, 1000 mg/kg was lethal.

(60)

(2)

Type: Micronucleus assay (mouse peripheral blood)
Species: Mouse
Sex: male/female
Strain: B6C3F1
Route of admin.: oral feed (microencapsule)
Exposure period: 14 weeks
Doses: 0, 3900, 7800, 15600, 31300 ppm (average daily doses: See Remarks)
Result: No increases in the frequency of micronucleated erythrocytes were observed in peripherial blood samples collected frome male and female mice with in 24 hour of the final exposure in the 14-week study (See the section 5.4).
Method: other: no data
Year: **GLP:** no data
Test substance: purity: 96.5 % (mixture of two geometric isomers: 2:1 geranial:neral)
Remark: Sampling was conducted from peripheral blood within 24 hours after 14-week feeding study (See the section 5.4).
Dietary concentrations of 3900, 7800, 15600 and 31300 ppm resulted in average daily doses of approximately 745, 1840, 3915 and 8110 mg/kg to males and 790, 1820, 3870 and 7550 mg/kg to females.

(60)

5.7 Carcinogenicity

(1)

Species: Rat
Sex: male/female
Strain: F344/N
Route of admin.: oral feed (microcapsule)
Exposure period: 2 years
Frequency of treatment: daily
Post. obs. period: none
Doses: 1000, 2000, 4000 ppm (daily intake: approximately 50, 100, 210 mg/kg/day)
Result:
Control Group: untreated and vehicle control
Method: other
Year: **GLP:** no
Test substance: purity: 94 % (an isomer composition of approximately 63 % geranial and 37 % neral)
Result: Survival of all exposed groups of males was significantly greater than that of the vehicle control group. Mean body weights of rats exposed to 4000 ppm were generally less than those of the vehicle control groups from week 49 (males) or 25 (females) to the end of the study., Feed consumption by exposed groups was similar to that by the vehicle controls.
There was the significantly increased incidence of mononuclear cell leukemiain in 1000 ppm females (vehicle, 10/50; 1000 ppm, 22/50; 2000 ppm, 14/50; 4000 ppm, 15/50), which was not considered to be treatement-related because of no obvious exposure concentration-related response. The incidence (42/50, 45/50, 48/50, 50/50) and severity of kidney mineralization was increased concentration-relatedly in males. Because the vehicle control incidence of this change was 84 %, the increased incidences observed in the exposed groups are believed to reflect an exacerbation of spontaneously occurring lesion. A significant increase in the incidence of adrenal cortical angiectasis (1/50, 1/50, 3/50, 10/50) is considered to be within the normal range of histopathological lesion observed in aged rats.

(60)

(2)

Species: Mouse
Sex: male/female
Strain: B6C3F1
Route of admin.: oral feed (microcapsule)
Exposure period: 2 years
Frequency of treatment: daily
Post. obs. period: none
Doses: 500, 1000, 2000 ppm (daily intake: approximately 60, 120, 260 mg/kg/day)
Result:
Control Group: untreated and vehicle control
Method: other
Year: **GLP:** no
Test substance: purity: 94 % (an isomer composition of approximately 63 % geranial and 37 % neral)
Result: Survival of exposed males and females groups was similar to that of the vehicle control group. Mean body weights of mice exposed to 1000 or 2000 ppm were generally less than those of the

vehicle controls throughout the study, and mean body weights of 500 ppm females were less from week 30 to the end of the study. Feed consumption by exposed groups was similar to that by the vehicle controls.
The incidences of malignant lymphomain females occurred with a positive trend (vehicle, 4/50; 500 ppm, 3/49; 1000 ppm, 5/50; 2000 ppm, 12/50). The incidence in 2000 ppm females was significantly greater than that in the vehicle control group. The tissue most commonly affected by malignant lymphoma were the spleen, mesenteric lympho node, thymus and, to lesser extent, ovary. There was a significant positive trend in the incidence of hepatocellulat adenoma in females (3/49, 2/50, 8/50, 8/50), but neither pairwise comparisons for hepatocellular adenoma nor trends for hepatocellular carcinoma or hepatocellular adenoma of caucinoma (combined) were significant. Inflammation and ulceration fothe oral mucosa were present in all groups of mice. The incidences of inflammation in 2000 ppm males (12/50, 16/50, 21/50, 10/50) and inflammation and ulceration in all groups of exposed females (14/49, 32/50, 35/50, 32/50) were significantly increased. The inflammation and ulceration are considered to be secondary to embedded hair shafts either present in the actual section or in an adjacent plane of section. These same lesions, with similar severity, were observedin vehicle controls. The incidences of bone fibrosis were significantly increased in 500 and 1000 in females (11/49, 22/50, 21/50, 18/50). The incidences of adrenal cortical focal hyperplasia were increased in exposed males versus the vehicle controls, and the increase was significant in the 2000 ppm group (0/50, 3/50, 1/50, 5/50). There was an exacerbation of minimal nephropathy in exposed females, and the incidence was significantly increased in the 2000 ppm group (9/49, 16/50, 15/50, 17/50). The 500 and 1000 ppm females had significantly increased incidences of minimal renal tubule mineralization compared to that in the vehicle controls (4/49, 14/50, 18/50, 6/50).

(60)

5.8 Toxicity to Reproduction

(1)

Species: rat **Sex:** male/female
Strain: SD (Crj(CD))
Route of admin.: gavage
Exposure period: Male: 46 days, Female: 39-50 days from before mating to day 3 of lactation,throughout gestation period
Frequency of treatment: daily
Post. obs. period: no
Doses: 40, 200, 1000 mg/kg
Control Group: yes, concurrent no treatment (corn oil)
NOAEL: 1,000 mg/kg
Method: OECD Preliminary Reproduction Toxicity Screening Test (TG 421)
Year: 1996 **GLP:** yes
Test substance: purity, 98.20%
Result: No effects of citral were detected in reproductive ability, organ weights or histopathology of the reproductive organs of both sexes, delivery or matrnal behaivor of dams. Male and female pups of the 1000 mg/kg group showed a lower body weight. No effects of citral were revealed in viability, general appearance or autopsy of pups.

(56)

(2)

Type: Fertility
Species: rat **Sex:** female
Strain: Wistar
Route of admin: dermal
Exposure Period: 60 bzw. 100 Tage
Frequency of treatment: taeglich
Premating Exposure Period female: 60 bzw. 100 Tage
Duration of test: eine Angaben
Doses: 460 mg/kg in 70 % Ethanol (50 ul)
Control Group: yes, concurrent vehicle
Method: other
Year: **GLP:** no data
Test substance: purity: unknown
Result: Waehrend der Versuchsdauer wurden keine toxischen Veraenderungen bei den Tieren festgestellt. Nach Behandlungsende wurde ein Teil der Tiere getoeten und untersucht, ein Teil wurde mit maennlichen Tieren gepaart. Sowohl die Behandlung ueber 60 wie auch 100 Tage fuehrte zu einer Reduktion der Implantationen und Nachkommen. Der Postimplantationsverlust betrug 28 % in der 60 Tage Gruppe und 31,8 % in der 100 Tage Gruppe (Kontrolle 7.4 %). Alle Nachkommen der Tiere, die 100 Tage behandelt wurden starben kurz nach der Geburt. Kein Effekt auf das Koerpergewicht der Nachkommen wurde beschrieben. Die Ovarien der behandelten Tiere waren kleiner als die von Kontrolltieren. Die Zahl der urspruenglichen primaeren Follikel war reduziert. Waehrend die 60 taegige Behandlung keinen Effekt auf die Zahl der Corpora lutea hatte, war die Zahl der Corpora lutea bei Tieren die 100 Tage behandelt wurden signifikant reduziert. Aufgrund des limitierten Untersuchungsumfangs erscheinen die Ergebnisse als nicht plausibel, die Studie entspricht nicht den heutigen Guidelines. Die biologische Relevanz ist daher nicht zu bewerten.
Source: BASF AG Ludwigshafen

(67)

(3)

Type: Fertility
Species: rat **Sex:** female
Strain: Wistar
Route of admin.: i.p.
Exposure Period: 6 Oestruszyklen
Frequency of treatment: Tag des Pro-Oestrus, in 6 aufeinanderfolgenden Oestruszyklen
Duration of test:
Doses: 460 mg/kg
Control Group: yes
Method: other
Year: **GLP:** no data
Test substance: purity: unknown
Result: Waehrend der Behandlungsdauer wurden keine toxischen Effekte festgestellt. Die Haelfte der Tiere (n=14) wurde nach der Behandlung getoetet und untersucht, die andere Haelfte (n=14) wurde verpaart.
Ein erhoehter Postimplantationsverlust (17.1 %) und eine verringerte Zahl von Nachkommen wurde beschrieben.
Bei den behandelten Tieren wurden verkleinerte Ovarien festgestellt, wie auch eine verringerte Zahl urspruenglicher primaerer Follikel.

Die Ergebnisse dieser Studie sind aufgrund der unphysiologischen Applikation und des limitierten Versuchsumfangs nicht zu bewerten. Die Ergebnisse erscheinen als nicht plausibel.

Source: BASF AG Ludwigshafen

(68)

(4)

Type:
Species: mouse **Sex:** female
Strain: CD-1
Route of admin.: gavage
Exposure Period: 6. - 15. Tag der Traechtigkeit
Frequency of treatment: keine Angaben
Duration of test: keine Angaben
Doses: keine Angaben
Control Group: no data specified
Method: other: no data
Year: **GLP:** no data
Test substance: purity: unknown
Remark: Status einer NTP-Reprotox.-Studie: "cancelled".
Source: BASF AG Ludwigshafen

(69)

5.9 Developmental Toxicity/Teratogenicity

(1)

Species: rat **Sex:** male/female
Strain: SD (Crj(CD))
Route of admin.: gavage
Exposure period: Male: 46 days, Female: 39-50 days from before mating to day 3 of lactation, throughout gestation period
Frequency of treatment: daily
Post. obs. period: no
Doses: 40, 200, 1000 mg/kg
Control Group: yes, concurrent no treatment (corn oil)
NOAEL: 200 mg/kg
Method: OECD Preliminary Reproduction Toxicity Screening Test (TG 421)
Year: 1996 **GLP:** yes
Test substance: purity, 98.20%
Result: Male and female pups of the 1000 mg/kg group showed a lower body weight. No effects of citral were revealed in viability, general appearance or autopsy of pups.

(56)

(2)

Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: 6. - 15. Tag der Traechtigkeit
Frequency of treatment: taeglich, 6 Stunden pro Tag
Duration of test: 20 days
Doses: 0.06, 0.21 mg/l (10, 34 ppm; Dampf), 0.43 mg/l (68 ppm; Aerosol-Dampf-Gemisch)
Control Group: yes, concurrent vehicle

NOAEL Maternalt.: .21 mg/l
NOAEL Teratogen.: .43 mg/l
Method: other
Year: **GLP:** no data
Test substance: purity: unknown
Result: In der hoechsten Dosisgruppe wurde maternale Toxizitaet beobachtet, nicht jedoch in den beiden niedrigeren Dosisgruppen. In der hoechsten Dosisgruppe wurden verminderte Koerpergewichtszunahme, Truebung der Augen, Atemschwierigkeiten (bis zum 20. Tag der Traechtigkeit), Nasenausfluss und vermehrter Speichelfluss festgestellt. Es wurden keine statistisch signifikanten Abweichungen bei der Zahl der Corpora lutea, der Implantationen und der postimplantaeren Verluste zwischen den exponierten und den Kontrolltieren beobachtet. In keiner Gruppe wurden tote Feten festgestellt. Obwohl die Zahl der Praeimplantationsverluste bei den Citral-exponierten Tieren etwas hoeher lag,waren die Wurfgroessen nicht signifikant vermindert. Die beiden Feten der Citral-behandelten Tiere beobachteten Missbildungen (visceral und skelettal) waren statistisch nicht signifikantunterschiedlich im Vergleich zu den Feten der Kontrolltiere.Die Autoren bewerteten die Testsubstanz unter den gewaehltenBedingungen bis zur hoechsten geprueften Konzentration, die bereits maternal toxisch wirkte, als nicht teratogen.
Source: BASF AG Ludwigshafen

(70)

(3)

Species: rat **Sex:** female
Strain: Wistar
Route of admin.: gavage
Exposure period: 9 days from day 6 to 15 of pregnancy
Frequency of treatment: daily
Duration of test:
Doses: 60; 125; 250; 500 and 1000 mg/kg
Control Group: yes, concurrent vehicle
NOAEL Embryofetus.: 125 mg/kg
Method:
Year: **GLP:** no data
Test substance:
Remark: Caesarean sections were carried out on day 21 of pregnancy, and the number of resorptions and implantation sites were recorded. Fetuses were weighed, examined for external malformations, and fixed for visceral examination, or cleared and stained with Alizarin Red S for skeleton evaluation.
Result: A transient decrease in weight gain from days 6 to 11 of gestation at the lowest doses, and a reduction in body weight minus uterine weight at term at the highest doses, indicated that citral was maternally toxic over the dose range tested. A slight but statistically significant increase in the ratio of resorptions per implantations was observed with 60 and 125 mg/kg body weight. Doses higher than 125 mg/kg reduced dose-dependently the ratio of pregnant per mated female. Signs of fetal growth retardation and a higher incidence of minor skeletal abnormalities were found in doses higher than 60 mg/kg. No increase in the frequency of visceral anomalies was found at any dose level, but an increase in fetal spleen weight was observed in doses higher than 125 mg/kg. Therefore, data presented in this paper indicate that the no-observed adverse effect level for embryofeto-toxicity is lower than 60 mg citral/kg body weight
Source: BASF AG Ludwigshafen

(71)

(4)

Species: rat **Sex:** female
Strain: no data
Route of admin.: i.p.
Exposure period: 10. bis 12. Tag der Traechtigkeit
Frequency of treatment: taeglich
Duration of test: keine Angaben
Doses: 360 mg/kg
Control Group: no data specified
Method: other: no data
Year: **GLP:** no data
Test substance: purity: unknown
Remark: Maternale Tox.: keine Angaben
Result: Bei intraperitonealer Verabfolgung wurden keine embryotoxischen Effekte beobachtet. Die Autoren halten den Abbau von Citral durch Detoxifizierungsmechanismen dafuer verantwortlich. Um diese zu umgehen wurden auch Versuche mitintraamnionaerer Gabe (15 ug/embryo) durchgefuehrt. Dies fuehrte bei 80 % der Embryonen zu Letalitaet. Die Aussagekraft des zweiten Versuches ist aufgrund voellig unphysiologischer und irrelevanter Exposition nicht zu beurteilen.
Source: BASF AG Ludwigshafen

(72)

5.10 Other Relevant Information

(1)

Type: Metabolism
Remark: Hepatic mitochondrial and cytosolic fractions were prepared from male SD rats to assess in vitro metabolism of citral. Evidence of aldehyde dehydrogenase- mediated citral oxidation was not seen in either subcellular fraction. On the contrary, citral was found to be a potent inhibitor of acetaldehyde oxidation by the low-Km mitochondrial form of aldehyde dehydrogenases. Measurement of the in vitro acetaldehyde oxidation rates of this isozyme in the presence of citral lead to the estimation of a Ki of 360 nM. Further studies of citral effects on low-Km mitochondrial aldehyde dehydrogenases indicate that inhibition is by a linear mixed-type mechanism and does not involve citral binding at the NAD+ binding site. In addition, it was observed that citral was readily reduced to the corresponding alcohol by alcohol dehydrogenases in the cytosolic fraction. The reduction of citral in the presence of NADH procceeded at two distinct rates; an initial “fast” rate followed by a “slow” rate. Individual kinetic constants were calculated for the two rates. It is possible that the differential alcohol dehydrogenase-mediated reduction rates of citral are the result of varying affinities for the enzyme of the two citral isomers, geranial (trans) and neral (cis). Bases on these findings it seems unlikely that direct aldehyde dehydrogernases-mediated oxidation of citral is involved in its in vivo metabolism, but rather that citral may undergo an alcohol dehydrogenases –mediated reduction to

the corresponding alcohol or metabolism via other pathways prior to oxidation by aldehyde dehydrogenases.

Source: BASF AG Ludwigshafen

(73)

(2)

Type: Metabolism

Remark: Bestimmung der Metaboliten versch. Terpenoide, u.a. Citral, im Urin beim Kaninchen. Aus dem Isomerengemisch von Citral (aus Geranial und Neral, 1:1) ergaben sich etwa 10 Stoffwechselprodukte. Eines davon wurde als (2E, 6E)-3,7-dimethyl-2,6-octadien-1,8-disaeure identifiziert.

Source: BASF AG Ludwigshafen

(74)

(3)

Type: Metabolism

Remark: For metabolite identification, urine was collected from F344 rats 24 hr after a single po 500 mg/kg dose of [14C] citral. Citral was rapidly metabolized and excreted as metabolites, including several acids and a biliary glucuronide. Seven urinary metabolites were isolated and identified: 3-hydroxy-3,7-dimethyl- 6-octenedioic acid; 3,8-dihydroxy-3,7-dimethyl-6-octenoic acid; 3,9-dihydroxy-3,7-dimethyl-6-octenoic acid; *E*- and *Z*-3,7-dimethyl-2,6-octadienedioic acid; 3,7-dimethyl-6-octenedioic acid; and *E*-3,7-dimethyl-2,6-octadienoic acid. Although citral is an alpha, beta-unsaturated aldehyde and has the potential of being reactive, the urinary metabolites of citral appear to arise from metabolic pathways other than nucleophilic addition to the double bond.

Source: BASF AG Ludwigshafen

(75)

(4)

Type: Distribution

Remark: Mit 14C-markiertem Citral wurde die Auswirkung einer gleichzeitigen Gabe von Limonol auf die Verteilung in Hautschichten und Proteinfraktionen untersucht.

Source: BASF AG Ludwigshafen

Test substance: Citral, Limonen

(76)

(5)

Type: Toxicokinetics

Remark: Metabolism data are reported in the literature for rats. The disposition of [^{14}C] citral was studied in male Fischer rats after iv with 5 mg/kg, po with 5, 50, or 500 mg/kg, and dermal treatments with 5 or 50 mg/kg. At 72 hr after exposure, rats were sacrificed and tissues were analyzed for ^{14}C. The amount of ^{14}C found in any tissue was a very small percentage (< 2%) of the total dose. The relative amount of radioactivity in all tissues did not change with increasing dose or route of exposure. The pattern of distribution and elimination was the same after iv or oral exposure. Urine was the major route of elimination of [^{14}C] citral, followed by feces, $^{14}CO_2$ (*via* lung), and expired volatiles. However, after dermal exposure, relatively less of the material was eliminated

in the urine and more in the feces, suggesting a role for first-pass metabolism through the skin. Citral was almost completely absorbed orally; due to its extreme volatility, much of an applied dermal dose was lost. The citral remaining on the skin was fairly well absorbed. Although the feces were a minor route of excretion, approximately 25% of the administered dose was eliminated *via* the bile within 4 hr of an iv dose. The metabolism of citral was both rapid and extensive. Within 5 min of an iv dose, no unmetabolized citral could be detected in the blood. Repeated exposure to citral resulted in an increase in biliary elimination, without any significant change in the pattern of urinary, fecal, or exhaled excretion. This suggestes that citral may induce at least one pathway of its own metabolism. The rapid metabolism and excretion of this compound suggest that significant bioaccumulation of citral would not occur.

Source: BASF AG Ludwigshafen

(77)

(6)

Type: Toxicokinetics

Remark: The absorption, tissue, distribution, and excretion of ^{14}C labeled citral has been investigated in the rats and mice. In both species, orally administered citral was rapidly absorbed from the gastro-intestinal tract, resulting in a fairly uniform distribution of label throughout the body of the mouse by 12 hr. In both species, radioactivity was excreted rapidly, the major route being the urinary tract. A significant propotion of the radioactivity was converted to $^{14}CO_2$ by the rat. Most of the administered radioactivity was excreted within 72 hr by the rat and within 120 hr by the mouse. There was no evidence for long-term storage of citral in the body, and it is suggested that any hazard associated with tissue accumulation after prolonged exposure will be minial.

Source: BASF AG Ludwigshafen

(78)

(7)

Type: Anti-promoting activity

Remarks: Citral was tested its ability to modulate tumor promotion in a two-stage skin-carcinogenesis study in hairless mice. The dorsal skins of female skh/hr1 mice were initiated with 0.1 umol dimethylbenzanthracene, and tumors were promoted by twice-weekly application of 10 nmol of tetradecanionyl-phorbol- 13-acetate(TPA) for 20 weeks. Prior to each TPA application groups were dosed with 0, 1 or 10 umol citral. Citral had a dose-dependent inhibitory effect on tumor-production in the TPA promoted groups.

Source: BASF AG Ludwigshafen

(79)

(8)

Type: A weak peroxisome proliferating activity

Remarks: Wistar or Long Evans were administered by gavage with citral at 2400 mg/kg for 3 or 10 days. In both straines, biochemical examination revealed changes of liver enzymes, and pahtological examinations revealed increases in liver weight (absolute and relative) with the perivascular loss of glycogen and the distribution of neutral fat altered

with periportal and mid-zonal accumulation of lipid droplets. Specific biochemical marker supported the morphological changes in the peroxisomes. Cyanide-insensitive palmitoyl CoA oxidation showed, at the maximum, fourfold and threefold inductions in Wistar albino and Long Evans hooded rats, respectively. In addition, induction of cytochrome P-450 levels was greater in the Long Evans than in the Wister rats, the maximal increases recorded being 81 and 27% respectively. A peroxisome-associated polypeptide of molecular weight 80,000 daltons (PPA-80) was induced, especially in Long Evans rats. The study indicates that peroxisomal and possibly also mitochondrial changes are involved in the action of citral on lipid metabolism.

Source: BASF AG Ludwigshafen

(80)

(9)

Type: A weak peroxisome proliferating activity

Remarks: Male Wistar rats were administered by gavage with citral at 1500 mg/kg for 5 days. Citral caused peroxisome proliferation as indicated by induction of cyanide-insenitive palmitoyl-CoA oxidant and bifunctional enzyme; levels of microsomal cytochrome P-450 IVA1 were also raised.

Source: BASF AG Ludwigshafen

(81)

(10)

Type: Prostate glands proliferating activity

Remarks: Typical lesions of benign prostatic hyperplasia (BPH) were induced in the ventral prostate of aldolescent male rats dermally treated at 150 mg/kg/day of citral up to 90 days. Incipient BPH changes were already observed in the acinar glands 10 days after citral administration. A longer period of treatment (1 month) significantly enhanced epithelial hyperplasia. Characteristic BPH lesions involving both prostatic compartments were found after 3 months of treatment. In rats castrated prior to administration, citral did not induce such BPH changes. Therefore, this BPH change induced by citral is considered to be dependent on the presence of the testicle.

Source: BASF AG Ludwigshafen

(82)

(11)

Type: Prostate glands proliferating activity

Remarks: Citral was treated dermally to male rats at 185 mg/kg for 30 days changing the administration schedule (every four days at 12 am instead of a daily one at 8 am) and the location of the application. This change in the experimental schedule induced relevant histological lesions of atypical acinar hyperplasia of the ventral prostate, instead of BPH as obtained by a daily citral treatment in a constant skin area. An increase of citral efficiency is likely due to an improvement of its absorption and lower testosterone level at noon than during the morning.

Source: BASF AG Ludwigshafen

(83)

(12)

Type: Prostate glands proliferating activity

Remarks: In male rats treated dermally with citral, marked hyperplasia of glandular epithelium and interglandular stroma were observed in the ventral prostate. Despite the cellular hyperplasia there was no significant increase in prostate weight. In immunocytochemical demonstration of BrdUrd incorporation, no significant changes in BrdUrd-positive cells either in glandular epithelium of in stroma within the ventral prostate could be detected. Investigations of the mechanism of action of citral showed that application of citral directly to the vagina in female, ovariectomized rats resulted in an increased proliferation of vagina epithelium and a significant increase in the BrdUrd incorporation of vaginal epithelial cells, in short a similar effect to that of estrogen application.
In steroid mesurement, there were no treatment-induced alterations in serum levels of testosterone and 17beta-estradiol in citral-treated male and female rats.
In an *in vitro* assay citral proved to inhibit estrogen binding to estrogen receptors, while no such inhibition was observed with testosterone for anrogen receptors.

Source: BASF AG Ludwigshafen

(84)

(13)

Type: Prostate glands proliferating activity

Remarks: The possible interactions between citral and serum testosterone levels on the induction of hyperplastic changes in the ventral prostate of adolescent rats were investigated. In addition, the study includes a comparative analysis of normal intact rats showing circadian variations of serum testosterone levels and rats in whom this rhythmic pattern was abolished either by excessive supplementation of exogenous androgen or by castration. The results demonstrate an induction of benign as well as atypical prostatic hyperplasia following citral application. In rats castrated prior to administration, citral did not induce such BPH (benign prostatic hyperplasia) changes. On the other hands, BPH was induced in castrated rats with testosterone implants. The most severe atypical changes were noted in the citral-treated rats with high serum testosterone levels.

Source: BASF AG Ludwigshafen

(85)

(14)

Type: Prostate glands proliferating activity

Remarks: Immunocytochemical characterization of several epithelial markers using the PAP technique was analyzed during different stages of induced prostatic hyperplasia in rats. Intact adolescent rats (42 days old) were treated with citral (3,7 dimethyl-2,6 octadienal) for 10, 30 and 100 days and their ventral prostate compared to untreated, matched-age animals. Among the epithelial markers studied the prostatic specific acid phosphatase was present in hyperplastic prostates of rats. The immunoreaction showed a fair correlation with the severity of lesion and duration of treatment. The prostatic specific antigen showed equally immunoreactive in both control and treated rats. The hyperplastic and normal rat prostates did not show immunoreactivity towards the other epithelial cell markers such as epithelial membrane antigen, carcinoembrionic antingen and alpha-fetoprotein antisera. It is concluded that prostatic specific acid phosphatase, and to a lesser extent prostatic

specific antigen, might represent valuable markers for comparative studies of prostatic hyperplasia in rodents.

Source: BASF AG Ludwigshafen

(86)

(15)

Type: Prediction of carcinogenesis by computer analysis

Remarks: A recent prediction for citral carcinogenicity made on the basis of computer tests (COMPACT: Computer Optimised Molecular Parametric Analysis for Chemical Toxicity) was negative.

Source: BASF AG Ludwigshafen

(87)

(16)

Type: Biochemical or cellular interactions

Remark: Citral inhibits the oxidation of retinol to retinoic acid in mouse epidermis n local application. This inhibitory property was used to the hypothesis that oxidation to retinoic acid is rate limiting for the biological activity of vitamin A(retinol) in epithelial tissue. Citral was tested as a modulator of the biological activities of retinal and retinoic acid using two bioassays of performed in *SKH/HR* (hairless) mice: (a) the ability to induce epidermal hyperplasia; (b) the ability to inhibit induction of epidermal ornithine decarboxylase activity by tumor promoters. Citral treatment inhibited the ability of retinol, but not of retinoic acid, to induce epidermal hyperplasia. Similarly, citral treatment decreaed the ability of retinol, but not of retinoic acid, to inhibit the induction of epidermal ornithine decarboxylase activity by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate. Although citral had little effect on epiderma ornithine decarboxylase activity when applied alone, it potetiated the induction of ornithine decarboxylase activity by 12-*O*- tetradecanoylphorbol-13-acetate. The ability of citral to inhibit retinoic acid formation from retinol and the specificity of citral for inhibition of the biological activities of retionol but not retinoic acid are evidence that oxidation retinoic acid is obligatory for the measured biological activity of retinol. Furthermore, the ability of citral to potentiate the induction of ornithine decarboxylase activity by 12-*O*-tetradecanoylphorbol-13-acetate suggests that modulation of the retinol oxidation pathway by such agents may enhance susceptibility to tumor promoters.

Source: BASF AG Ludwigshafen

(88)

(17)

Type: Biochemical or cellular interactions

Remark: Untersuchung der haemolyt. Wirkung von Citral in vitro.

Source: BASF AG Ludwigshafen

(89)

(18)

Type: Biochemical or cellular interactions

Remark: Untersuchung der Leberenzym-induzierenden Wirkung (ca. 25 %)von Citral bei neonatalen Ratten.

Source: BASF AG Ludwigshafen

(90)

(19)

Type: other: ultrastructual study
Remark: Topical application of citral on male rat skin induces hyperplasia of the sebaceous glands. Ultrastructual study showed that this hyperplasia is manefested by an increase in number of the partially differentiated cells. Because citral causes an increase in testosterone level, the auther concludes that the sebaceous gland hyperplasia is related to androgen activity.
Source: BASF AG Ludwigshafen
(91)

(20)

Type: Cytotoxicity
Remark: Zytotoxische Wirkung auf Hela-Zellen.
Source: BASF AG Ludwigshafen
(92)(93)

(21)

Type: Cytotoxicity
Remark: Es wird von einer UV-A-(315-400 nm)-Licht verstaerkten, sauerstoffabhaengigen Toxizitaet der Testsubstanz gegenueberverschiedenen Staemmen von Escherichia coli berichtet. E. coli Staemme, die ein Gen aufweisen, das zu einer Katalase Defizienz fuehrt (katF), reagierten auf die Behandlung mit Citral und UV-A bezueglich einer Inaktivierung sensibler, als Katalase profiziente Staemme (katF+). In vitro produzierte die Testsubstanz bei UV-A Bestrahlung Wasserstoffperoxid.
Source: BASF AG Ludwigshafen
(94)

(22)

Type: other
Remark: Fungistatische Wirkung
Source: BASF AG Ludwigshafen
(95)(96)(97)

(23)

Type: other
Remark: Hinweis auf eine Fuetterungsstudie mit mikroverkapseltem Citral.
Source: BASF AG Ludwigshafen
(98)

(24)

Type: other
Remark: Sensibilisierung:
Quencheffekt von d-Limonene auf Citral-Sensibilisierung
Source: BASF AG Ludwigshafen
(99)

(25)

Type: other: Embryotoxizitaet, Teratogenitaet (Huehnerembryonen)
Remark: Untersuchungen an Huehnerembryonen.
Source: BASF AG Ludwigshafen
(100)(101)(102)(103)(104)(105)(106)(107)(108)

(26)

Type: other: Human
Remark: Mensch; 21 Tage
Hautreizwirkung am Menschen ueber 21 Tage, versch. Loesungengetestet.
Source: BASF AG Ludwigshafen

(35)

(27)

Type: other: Human
Remark: Mensch
Hautreizung beim Menschen unter versch. Bedingungen.
Source: BASF AG Ludwigshafen

(36)(108)

(28)

Type: other: antineoplastische Wirkung
Remark: Citral besitzt keine antineoplastiche Wirkung auf Ehrlich Ascites-tumor, Maeusesarkom 37 oder Maeusesarkom 180.
Source: BASF AG Ludwigshafen

(110)

(29)

Type: other: zusammenfassende Darstellungen
Remark: Zusammenfassende Darstellungen
Source: BASF AG Ludwigshafen

(111)(112)(113)(114)(115)

(30)

Type: Uterotrophy test
Remark: Wistar rat was administered orally with 0, 300 and 1,000 mg/kg of citral FCC. Adiministration was conducted 3 times daily. On the basis of the uterus weights citral did not show an estrogen-like potential. A statistically significant decrease of the absolute uterus weights at 1,000 mg/kg was regarded as not biologically relevant.
The sensitivity of the rats used in this test has been proven by application of a known positive contral substance DES/DP: in comparison to the carrier control a theefold increase of absolute and relative uterus weights resulted.

(116)

5.11 Experience with Human Exposure

(1)

Remark: During an investigation of an outbreak of dermatitis following the introduction of a lemon-scented detergent, citral was shown by a patch tests to be a strong primary irritant if applied in association with heat. At 23 °C, synthetic citral and two other "pure" citral samples, all at 10 % in alcohol, produced numerous slight responses at 20 min, but these disappeared within 24-48 hr. At 43 °C, these samples produced a large number of pronounced reactions after 20 min, and the reactions persisted after

24-48 hr. The toxic nature of the response could be detected in biopsies taken as late as 48 hr after exposure. A sample of "essensses et fractiones citral" produced no ractions after 20 min-48 hr at 23 or 43 °C.

(109)

(2)

Remark: Positiver Patch-Test auf Citral bei einer Faru nach Umgang mit Schalen von Citrusfruechten.
Source: BASF AG Ludwigshafen

(117)

(3)

Remark: A survey of sensitisation data from tests on materials containing citral was conducted under the auspices of the Soap and Detergent Association (SDA). This survey was restricted to skin patch tests on human subjects conducted in the USA by member companies of SDA and by perfume suppliers. None of the personal care or household products containing citral induced hypersensitivity attributed to citral in 10,660 patch tests and there were no confirmed reactions to citral in 2,098 patch tests on fragrance blends containing the substance. A total of 22 induced sensitization occurred in 174 tests conducted at 1 to 5% pure citral in ethanol, but this effects appears to be dose related since no inductions occurred at the 0.5% citral concentration level in 82 test subjects.

Source: BASF AG Ludwigshafen

(118)

(4)

Remark: Modifizierter Draize-Test bei 0.1 % negativ, bei 0.4 % positiv.
Source: BASF AG Ludwigshafen

(119)

(5)

Remark: Fallbericht ueber positive Testreaktion auf Citral.
Source: BASF AG Ludwigshafen

(120)

(6)

Remark: Angaben ueber positive Testreaktionen bei 1.7 % von 228 Testpatienten.
Source: BASF AG Ludwigshafen

(121)

(7)

Remark: Marginale Hautreizung von 8 % Citral im kumulativen Patch-Test.
Starke Hautreizung von 10 % Citral bei Erhitzung auf 43 oC im Patch-Test.
Im Maximierungstest mit natuerlichem und reinem Citral (4-8 %) positive Testreaktionen bei 4-8 von 25 Probanden.
Bei 48 % von 40 Probanden positive Patch-Testreaktion auf 4 % Citral; Reizungen bei 8 %.

Positiver Patch-Test bei 6 von 56 Probanden mit 8 % Citral.

Source: BASF AG Ludwigshafen

(122)

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ROBUST STUDY SUMMARIES

PHYSICAL/CHEMICAL ELEMENTS

BOILING POINT

Test Substance
- Citral (CAS: 5392-40-5)
- Remarks: Source:

Method
- Method: Unknown
- GLP: Unknown
- Year: Unknown
- Remarks: None

Results
- Boiling point value: 226 - 228 °C
- Pressure: Unknown
- Decomposition: No
- Remarks: None

Conclusions
Boiling point is 226 - 228 °C.

Data quality
- Reliabilities: Key study
- Remarks:

References
BASF AG Ludwigshafen

Other
- Last changed:
- Order number for sorting
- Remarks:

VAPOR PRESSURE

Test Substance
- Citral (CAS: 5392-40-5)
- Remarks: source: Tokyo Kasei Kogyo Co., LTD., mixture of trans (geranial) and cis (neral) isomers Purity: >94.6% (geranial: 62.6%, neral: 32.0%), kept at 5 °C until use. The structure was identified by Infrared red spectroscopy.

Method
- Method: OECD TG104 (Static method)
- GLP: yes
- Year: 1994
- Remarks: Vapour pressure was measured in triplicate at 20, 30 and 40 °C.

Results
Vapour Pressure value: < 130 Pa (40°C)
- Decomposition: no
- Remarks: Vapour pressure was less than 130 Pa at all temperature.

Conclusions
- The vapour pressure at 40 °C is < 130 Pa.

Data Quality
- Reliabilities: reliable without restrictions
- Remarks: Well conducted study, carried out by Chemicals Inspection & Testing Institute, Japan

References (Free Text)

Other
- Last changed
- Order number for sorting
- Remarks:

PARTITION COEFFICIENT

Test Substance
- Citral (CAS: 5392-40-5)
- Remarks: source: Tokyo Kasei Kogyo Co., LTD., mixture of trans (geranial) and cis (neral) isomers Purity: >94.6% (geranial: 62.6%, neral: 32.0%), kept at 5 °C until use. The structure was identified by Infrared red spectroscopy.

Method
- Method: OECD TG 117 (HPLC method)
- GLP: yes
- Year: 1994
- Remarks: The partition coefficient was determined in duplicate at 25°C with a 25:75 (v/v) methanol-water mixture as an eluent by using acetanilide, acetophenone, methyl benzoate, ethyl benzoate, phenyl benzoate and benzyl benzoate as reference compounds. The partition coefficients of two isomers were simultaneously determined. The regression linear equations between log k and log P for two isomers were calculated by least squares method.
-

Results
- Log P_{ow}: 2.8 (neral), 3.0 (geranial)
- Temperature: 25°C
- Remarks:

The regression lines of log k versus log P, the average retention times and log k for two isomers:

Isomer	Regression lines	Retention time (min)	log k
Geranial	logPow = 2.601 log k + 2.337 (r=0.983)	4.480	0.243
Neral	logPow = 2.601 log k + 2.337 (r=0.983)	4.125	0.185

Conclusions
- Log P_{ow} is 2.8 for neral and 3.0 for geranial.

Data Quality
- Reliabilities: reliable without restrictions, Key study
- Remarks: Well conducted study, carried out by Chemicals Inspection & Testing Institute, Japan

References (Free Text)

Other
- Last changed
- Order number for sorting
- Remarks:

WATER SOLUBILITY

Test Substance
- Citral (CAS: 5392-40-5)
- Remarks: source: Tokyo Kasei Kogyo Co., LTD., mixture of trans (geranial) and cis (neral) isomers Purity: >94.6% (geranial: 62.6%, neral: 32.0%), kept at 5 °C until use. The structure was identified by Infrared red spectroscopy.

Method
- Method: OECD TG 105 (Flask method)
- GLP: No
- Year: 2000
- Remarks: 1 g of the test substance was added in triplicate to 10 ml of water in glass vessel. The vessel was tightly stopped and then shook at 30 °C for 24 hours and then equilibrated for 24 hours at 25 °C with occasional shaking. After the aqueous phase was centrifuged, the concentration of the test substance was analysed with HPLC.

Results
- Value:

Time (hour)	Concentration of Citral (mg/L)	Average (mg/L)	pH
24	6.4×10^2		4.2
	6.3×10^2	6.4×10^2	4.1
48	6.0×10^2		3.9
	6.1×10^2	6.0×10^2	3.9
72	5.4×10^2		3.7
	5.4×10^2	5.4×10^2	3.7

Total average of the concentration is 5.9×10^2 mg/L

- Description of solubility:
- pH value: Described in the Table.
- pKa value: There is no pertinent function group
- Remarks: A part of the test substance was converted to 6-methyl-5-heptene-2-on and geranic acid during the operation.

Conclusions
- Water solubility of Citral is 5.9×10^2 mg/L.

Data Quality
- Reliabilities: reliable with restrictions
- Remarks: Well conducted study, carried out by Chemicals Inspection & Testing Institute, Japan

References (Free Text)

Other
Remarks: This value (590 mg/L) is close to the result by BASF which is 420 mg/L.

STABILITY IN WATER

Test Substance
- Citral (CAS: 5392-40-5)
- Remarks: source: Tokyo Kasei Kogyo Co., LTD., mixture of trans (geranial) and cis (neral) isomers Purity: >94.6% (geranial: 62.6%, neral:32.0%), kept at 5 °C until use. The structure was identified by Infrared red spectroscopy.

Method
- Method/guideline: OECD TG 111
- Type: Hydrolysis as a function of pH
- GLP: yes
- Year: 1994
- Remarks: One mL of 2000 mg/L citral in methanol was added to 100 mL of each buffer of pH 4.0, 7.0 and 9.0. The test solutions was themostated in duplicate at 70, 80 and 90 °C and the concentration of the test substance was determined as a function of time with GC. The first-order rate constants of the hydrolysis for nine conditions were estimated by lease squares method. The rate constant of hydrolysis at 25°C was calculated by extrapolating the linear regression equation between the logarithm of the rate constant and reciprocal of temperature. The half-life time of hydrolysis was calculated from the rate constant.

Results
- Nominal concentration: 20 mg/L
- Measured value:
- Half-life ($t_{1/2}$) in days at pH 4.0, 7.0 and 9.0 extrapolated to 25 °C:

Isomer	pH 4.0	pH 7.0	pH 9.0
Geranial	9.81	106	22.8
Neral	9.54	230	30.1

- Breakdown products: not studied
- Remarks: The variation in the concentration of the test substance with time satisfactorily obeyed the first-order kinetics at all temperature

Conclusions
- Citral is hydrolysed in water. The rate of the hydrolysed is dependent on pH and different between geometric isomers.

Data Quality
- Reliabilities: reliable without restrictions, Key study
- Remarks: Well conducted study, carried out by Chemicals Inspection & Testing Institute, Japan

References (Free Text)

Other
- Last changed
- Order number for sorting
- Remarks:

TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS (FUGACITY)

Test Substance
- Citral (CAS No 5392-40-5)
- Remarks:

Method
- Test (test type): Calculation
- Method: Fugacity level III
- Year: 2001
- Remarks:

Results
- Media: air, water, soil and sediment
- Estimated Distribution and Media Concentration under three emission scenarios:

Compartment	Release 100% to air	Release 100% to water	Release 100% to soil
Air	97.7 %	1.7 %	0.1 %
Water	1.6 %	97.0 %	0.2 %
Soil	0.7 %	0.0 %	99.7 %
Sediment	0.0 %	1.3 %	0.0 %

- Remarks: Concerning the vapour pressure, calculated values by QSAR was used for this fugacity calculation (12.1 Pa as a vapour pressure), since we have only the range of this property (< 130 Pa as vapour pressure).

Conclusions
- If citral is released to one of the compartments of Air, Water and Soil, it is unlikely to distribute into other compartments.

Data Quality
- Reliabilities: Reliable without restrictions, Key study
- Remarks:

References (Free Text)
MITI, Japan

Other
- Last changed
- Order number for sorting
- Remarks:

BIODEGRADATION

Test Substance

- Citral (CAS: 5392-40-5)
- Remarks: source: Tokyo Kasei Kogyo Co., LTD., mixture of trans (geranial) and cis (neral) isomers - Purity: >97.5% (geranial: 64.1%, neral: 33.4%), kept at 5 °C until use. The structure was identified by Infrared red, mass and nmr spectroscopies.

Method

- Method/guideline: OECD TG 301C
- Test Type: aerobic
- GLP: yes
- Year: 1992
- Contact time: 28 days
- Inoculum: activated sludge cultivated for OECD TG 301C
- Remarks: 30 mg of the test substance or aniline (reference substance) and 9 mg as MLSS of the activated sludge were added to 300 mL of test medium. The test and reference solutions were cultivated in BOD meter together with the inoculum blank and abiotic control at 25°C for 28 days, during which the oxygen consumption was continuously measured. After termination of the test, the total organic carbon concentration (TOC) of the test solution and the residual amount of the test substance were determined with TOC meter and GC, respectively. The biodegradability was calculated from the oxygen consumption, TOC and the residual amount.

Results

- Degradation % after 28 days: 93, 88 and 94 % from BOD
  76, 76 and 82 % from TOC
  100, 100 and 100 % from GC
- Results: ready biodegradable
- Kinetic: Biodegradability of test substance from BOD: 9, 7 and 22 % after 7 days, and 69, 69 and 73 % after 14 days.
  Biodegradability of reference substance from BOD:65 % after 7 days, 87 % after 14 days and 102 % after 28 days
- Breakdown products: The test substance in the abiotic control was partly transformed into 6-methyl-5-heptene-2-on and geranic acid during the test.
- Remarks: The percent residue of the test substance in abiotic control after 28 days was 51 % in HPLC analysis, but 98 % of organic carbon of the test substance remained in abiotic control from TOC analysis and 6-methyl-5-heptene-2-on and geranic acid were observed with HPLC. These results indicate that the part of the test substance was transformed into these compounds in abiotic control without being ultimately biodegraded to carbon dioxide.

Conclusions

- This chemical is ready biodegradable

Data Quality

- Reliabilities: reliable without restrictions, Key study
- Remarks: Well conducted study, carried out by Chemicals Inspection & Testing Institute, Japan

References (Free Text)

ECOTOXICITY ELEMENTS

ACUTE TOXICITY TO FISH

Test Substance

- Identity: 3,7-Dimethyl-2,6-octadienal
- Remarks: Source: Wako Pure Chemical Industries - purity = 98.9 % (Lot. TWQ2420), no information on isomer ratio

Method

• **Method/guideline followed (experimental/calculated):** OECD Test Guideline 203 (1981)
• **Type (test type):** 96 hours mortality
• **GLP (Y/N):** No
• **Year (study performed):** 1992
• **Species/Strain/Supplier:** *Oryzias latipes* (Medaka), obtained from commercial hatcheries
• **Analytical monitoring:** No
• **Exposure period:** 96 hours
• **Statistical methods:** Probit method, 72h-LC50 and 96hLC50 were driven by geometric mean of the highest concentration producing no mortality and the lowest concentration producing 100 % mortality (OECD TG203).

=> Remarks field for Test Conditions:

– Test fish (Age/length/weight, loading, pretreatment): Acclimated for several days before testing; any groups showing > 5 % mortality during 7 days were not used for testing

– Test conditions, e.g.

· Details of test (static, semi-static, flow-through): Semi-static, closed-system
· Dilution water source: Purified tap water
· Dilution water chemistry (hardness, alkalinity, pH, DOC, TSS, salinity): Not described
· Stock and test solution and how they are prepared:
· Concentrations dosing rate, flow-through rate, in what medium: Concentrations of 1.0, 1.7, 3.1, 5.6 and 10 mg/L were tested, control with and without the vehicle were taken for test.
· Vehicle/solvent and concentrations: Final concentration of 550 mg/L DMSO was used.
· Stability of the test chemical solutions: Not described
· Exposure vessel type (e.g., size, headspace, sealed, aeration, lighting, # per treatment): Test vessel was sealed with a silicon rubber.
· Number of replicates, fish per replicate: 10 fish were used for each concentration.
· Water chemistry in test (O_2, pH) in the control and one concentration where effects were observed: Dissolved oxygen and pH were measured daily; pH 6.9 - 7.3, DO = 4.9 - 9.1 mg/L
· Test temperature range; 21 - 23 °C

– Method of calculating mean measured concentrations (i.e. arithmetic mean,

geometric mean, etc.): Not described

Results

- **Nominal concentrations (as mg/L):** 1.0, 1.7, 3.1, 5.6 and 10
- **Measured concentrations (as mg/L):** Not described
- **Unit (results expressed in what unit):** mg/L
- **Element value:** LC_{50} at 24 hours = 6.1 mg/L (95 % confidence limits: not available)
  LC_{50} at 48 hours = 5.8 mg/L (95 % confidence limits: not available)
  LC_{50} at 72 hours = 4.1 mg/L (95 % confidence limits: not available)
  LC_{50} at 96 hours = 4.1 mg/L (95 % confidence limits: not available)
- **Statistical results, as appropriate:** Not described

=> Remarks field for Results:

– Biological observations: Not described

– Table showing cumulative mortality:

Chemical concentration (mg/L)	Cumulative mortality of each exposure (%)			
	24 hours	48 hours	72 hours	96 hours
0	0	0	0	0
1.0	0	0	0	0
1.7	0	0	0	0
3.1	0	0	0	0
5.6	30	40	100	100
10	100	100	100	100

– Lowest test substance concentration causing 100 % mortality: at 5.6 mg/L (72 hours)

– Mortality of controls: 0 % during exposure period

– Abnormal responses: Not described

– Reference substances (if used) – results: LC_{50} (96 hours) = 0.32 mg/L for sodium pentachlorophenol

Conclusions

Data Quality

- **Reliabilities:** Klimisch Code: 2 = Reliable with restrictions

=> Remarks field for Data Reliability

References

Environment Agency Japan (1994): Investigation on the Ecotoxicological effects of OECD High Volume Chemicals (Phase 3): p. 167 - 208.

Other

- Last changed (administrative field for updating)
- Order number for sorting (administrative field)
- Remarks field for General Remarks (Use for any other comments necessary for clarification.)

TOXICITY TO AQUATIC PLANTS (E.G., ALGAE)

Test Substance

- Identity: 3,7-Dimethyl-2,6-octadienal
- Remarks: Source: Wako Pure Chemical Industries - purity = 98.9 % (Lot. TWQ2420), no information on isomer ratio

Method

- **Method/guideline followed (experimental/calculated):** OECD Test Guideline 201 (1984)
- **Test type (static/other):** Static, closed system
- **GLP (Y/N):** No
- **Year (study performed):** 1992 - 1993
- **Species/strain # and source:** *Selenastrum capricornutum*, ATCC22662
- **Element basis:** The area below growth curve
- **Exposure period:** 72 hours
- **Analytical monitoring:** No
- **Statistical methods:** Probit method

=> Remarks field for Test Conditions:

– Test organisms
  · Laboratory culture
  · Method of cultivation
  · Controls

– Test Conditions
  · Test temperature range: 23 - 25 °C
  · Growth/test medium: OECD medium
  · Shaking: Occasional shaking
  · Dilution water source: Not described
  · Exposure vessel type: 100 mL-medium in a 300 mL-Erlenmeyer flask with a silicon cap which allow ventilation; The vessel was sealed with a Parafilm.
  · Water chemistry in test (pH) in at least one replicate of each concentration (at start and end of the test): Not described
  · Stock solutions preparation: Stock solution was prepared with DMSO (Final concentration at tests: 550 mg/l). Controls with and without this vehicle were taken for test.
  · Light levels and quality during exposure: 4000 - 7000 lx, continuous

–Test design:
  · Number of replicates: 3 parallel runs for each nominal concentration.
  · Concentrations: 1, 1.7, 3.1, 5.6 and 10 mg/L
  · Initial cell number in cells/mL: 1.0×10^4

– Method of calculating mean measured concentrations (i.e. arithmetic mean, geometric mean, etc.): Not described

Results

- **Nominal concentrations in mg/L:** 1, 1.7, 3.1, 5.6 and 10

• **Measured concentrations in mg/L:** Not described
• **Unit [results expressed in what unit]:** mg/L
• **EC_{50} (72 hours)** = 5.0 mg/L
• **NOEC** = 3.1 mg/L (p 0.05)
• **Was control response satisfactory:** Yes (Growth curves indicated the logarithmic growth during the test.)
• **Statistical results, as appropriate:** Not described

=> Remarks field for Results:

– Biological observations
· Cell density at each flask at each measuring point:

Test substance concentration (mg/L)	Cell concentration for each exposure ($x10^4$ cells/mL)			
	0 hour	24 hours	48 hours	72 hours
0	1.0	6.2	34.0	101.0
1	1.0	4.7	33.0	95.0
1.7	1.0	4.7	32.0	114.0
3.1	1.0	4.7	31.0	107.0
5.6	1.0	3.1	14.0	25.0
10	1.0	1.0	1.0	1.0

· Growth curves: Logarithmic growth until end of the test

Conclusion

Data quality
- **Reliabilities:** Klimisch Code: 2 = Reliable with restrictions
- Remarks field for Data Reliability

Reference
Environment Agency Japan (1994): Investigation on the Ecotoxicological effects of OECD High Volume Chemicals (Phase 3): p. 1 - 64.

Other
- Last changed (administrative field for updating)
- Order number for sorting (administrative field)
- Remarks field for General Remarks (Use for any other comments necessary for clarification.)

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (E.G., DAPHNIA)

Test Substance

- Identity: 3,7-Dimethyl-2,6-octadienal
- Remarks: Source: Wako Pure Chemical Industries - purity = 98.9 % (Lot. TWQ2420), no information on isomer ratio

Method

• **Method/guideline:** OECD Test Guideline 202 (1984)
• **Test type:** 24 hours immobilization test
• **GLP (Y/N):** No
• **Year (study performed):** 1992
• **Analytical procedures:** Not described
• **Species/Strain:** *Daphnia magna*
• **Test details:** Static, closed system
• **Statistical methods:** Probit method

=> Remarks field for Test Conditions:

– Test organisms:
  · Source, supplier, any pretreatment, breeding method: supplied from National Institute for Environmental Science
  · Age at study initiation: < 24 hours old
  · Control group:
– Test conditions
  · Stock solutions preparation and stability: Stock solution was prepared with DMSO: HCO-40 = 9:1 (Final concentration at test: 1.8 – 18 mg/L).
  · Test temperature range: 21 - 23 ?
  · Exposure vessel type: 50 mL-test solution in a glass beaker covered with film, 4 beakers per treatment
  · Dilution water source: Reconstituted water
  · Dilution water chemistry: hardness = 250 mg/L; pH 7.8; Ca/Mg ratio = 6.56; Na/K ratio = 6.54; alkalinity = 40.2 mg/L
  · Lighting: Not described
  · Water chemistry in test: pH 7.4 - 7.8; DO = 7.5 - 8.0 mg/L
– Element (unit) basis: immobilization
–Test design: 20 Daphnia (4 replicates; 5 organisms in each of replicate) were proposed at each nominal concentration (1.8, 3.2, 5.6, 10 and 18 mg/L).
– Method of calculating mean measured concentrations: Not described
– Exposure period: 24 hours
– Analytical monitoring: Not described

Results

• **Nominal concentrations in mg/L:** 1.8, 3.2, 5.6, 10 and 18

• **Measured concentrations:** Not described
• **Unit [results expressed in what unit]:** mg/L
• **EC_{50} (24 hours)** = 10 mg/L
• **Statistical results, as appropriate:** Not described

=> Remarks field for Results.

– Biological observations

• Number immobilization as compared to the number exposed: The test was carried out with 20 Daphnia at start for each nominal concentration.

Chemical Concentration (mg/L)	Immobility Number	Immobility Rate (%)
0	0	0
1.8	1	5
3.2	0	0
5.6	1	5
10	7	35
18	20	100

• Concentration response with 95 % confidence limits: 9.0 - 12 mg/L
• Cumulative immobilization: Not described
• Was control response satisfactory: Yes

Conclusions

Data quality

• **Reliabilities:** Klimisch Code: 2 = Reliable with restrictions
=> Remarks field for Data Reliability

References

Environment Agency Japan (1994): Investigation on the Ecotoxicological effects of OECD High Volume Chemicals (Phase 3): p. 65 - 164.

Other

- Last changed (administrative field for updating)
- Order number for sorting (administrative field)
- Remarks field for General Remarks (Use for any other comments necessary for clarification.)

Test Substance
• Identity: 3,7-Dimethyl-2,6-octadienal

Method
• **Method/guideline:** Directive 84/449/EEC, C.2 "Acute toxicity for Daphnia"
• **Test type:** 48 hours immobilization test
• **GLP (Y/N):**
• **Year (study performed):**
• **Analytical procedures:** Not described
• **Species/Strain:** *Daphnia magna*
• **Test details:**
• **Statistical methods:**
=> Remarks field for Test Conditions: Geprueft mit Tween 80 als Loesungsvermittler

Results
• **Nominal concentrations in mg/L:**
• **Measured concentrations:** Not described
• **Unit [results expressed in what unit]:** mg/L
• **EC_{50} (48 hours)** = 7 mg/L
• **Statistical results, as appropriate:** Not described

=> Remarks field for Results.
• Concentration response with 95 % confidence limits:
• Cumulative immobilization: Not described
• Was control response satisfactory:

Conclusions

Data quality
• **Reliabilities:** Klimisch Code: 2 = Reliable with restrictions
=> Remarks field for Data Reliability: Details were not available.

Reference
BASF AG, Labor Oekologie; unveroeffentlichte Untersuchung, (0547/88)

Other
- Last changed (administrative field for updating)
- Order number for sorting (administrative field)
- Remarks field for General Remarks (Use for any other comments necessary for clarification.)

CHRONIC TOXICITY TO AQUATIC INVERTEBRATES (E.G., DAPHNIA)

Test Substance

- Identity: 3,7-Dimethyl-2,6-octadienal
- Remarks: Source: Wako Pure Chemical Industries – purity = 98.9 % (Lot. TWQ2420), no information on isomer ratio

Method

• **Method/guideline:** OECD Test Guideline 202 (1984)
• **Test type:** 21 days reproduction test
• **GLP (Y/N):** No
• **Year (study performed):** Not described (1992 - 1993)
• **Analytical procedures:** Not described
• **Species/Strain:** *Daphnia magna*
• **Test details:** Semi-static (water renewal: 48 hours), closed system
• **Statistical methods:** Probit method for EC50 (reproduction), for LOEC not described.

Test Conditions:

– Test organisms:
  · Source, supplier, any pretreatment, breeding method: supplied from National Institute for Environmental Science
  · Age at study initiation: < 24 hours old
  · Control group:
– Test conditions
  · Stock solutions preparation and stability: Stock solution was prepared with DMSO: HCO-40 = 9:1 (Final concentration at test: 1.0 - 10 mg/L).
  · Test temperature range: 21 - 23 ?
  · Exposure vessel type: 400 mL-test solution in a 500 mL-glass beaker; 4 beakers per treatment
  · Dilution water source: Reconstituted water
  · Dilution water chemistry: hardness = 250 mg/L; pH 7.8; Ca/Mg ratio = 6.56; Na/K ratio = 6.54; alkalinity = 40.2 mg/L
  · Lighting: 16:8 hours; light-darkness cycle
  · Water chemistry in test: DO and pH were measured when the test solution was exchanged. The results of the measurement were not described.
  · Feeding: Green algae, daily
– Element (unit) basis: Reproduction
–Test design: 40 Daphnia (4 replicates: 10 organisms per replicate) were exposed to 5 nominal concentrations (1.0, 1.8, 3.2, 5.6 and 10 mg/L).
– Method of calculating mean measured concentrations (i.e. arithmetic mean, geometric mean, etc.): Not described
– Exposure period: 21 days
– Analytical monitoring: Not described

Results

• **Nominal concentrations:** 1.0, 1.8, 3.2, 5.6 and 10 mg/L

• **Measured concentrations:** Not described

• **Unit [results expressed in what unit]:** Mean cumulative numbers of juveniles produced per adult (reproduction)

• **Reproduction** EC_{50} (14 days) = 1.5 mg/L (95 % confidence limits: not available)
EC_{50} (21 days) = 1.6 mg/L (95 % confidence limits: not available)
NOEC = 1.0 mg/L
LOEC = 1.8 mg/L ($p < 0.05$)

• **Statistical results, as appropriate:** Not described

=> Remarks field for Results.

– Biological observations

• Time of the first production of young (d): 8 - 12 days

• Mean cumulative numbers of young produced per adult:

Chemical Concentration (mg/L)	Mean cumulative numbers of young produced per adult	
	14 days	21 days
0	24.5	57.5
1.0	24.8	63.8
1.8	1.6	11.4
3.2	0	0
5.6	0	0
10	0	0

• Was control response satisfactory: Yes

Conclusions

Data quality

• **Reliabilities:** Klimisch Code: 2 = Reliable with restrictions

=> Remarks field for Data Reliability: **Key study**

Reference

Environment Agency Japan (1994): Investigation on the Ecotoxicological effects of OECD High Volume Chemicals (Phase 3): p. 65 - 164.

Other

• Last changed (administrative field for updating)

• Order number for sorting (administrative field)

=> Remarks field for General Remarks (Use for any other comments necessary for clarification.)

HEALTH ELEMENTS

REPEATED DOSE TOXICITY (1)

Test Substance

- Citral (CAS No 5392-40-5)

Remarks: Source: Givaudan Corp.(Clifton, NJ, USA), Purity: mixture of two geometric isomers (approx, 70% geranial and approx. 30% neral)

Method

- **Method/guideline:** Unknown
- **Test type:** 14 days repeat oral dose toxicity test
- **GLP:** Unknown
- **Year:** 1993
- **Species:** Rat
- **Strain:** F344/N
- **Route of administration:** Oral by microencapsulation in feed
- **Doses/concentration levels:** 0, 0.63, 1.25, 2.5, 5, 10% citral microencapsulation (resultant doses in feeding :0, 142, 285, 570, 1140, 2280 mg/kg)
- **Sex:** Male & Female
- **Exposure period:** For 14 days
- **Frequency of treatment:** 7 days/week
- **Control group and treatment:** Concurrent vehicle (feed without microencapsules)
- **Post exposure observation period**: None
- **Duration of test:** For 14 days
- **Statistical methods:** Williames and Dunnett's test for continuous data

Test Conditions

- **Test Subjects:**
  - ***Age at study initiation:*** 28 days old for both sexes
  - ***Weight at study initiation*****:** About 95g for males, about 83g for females
  - **No. of animals per sex per dose:** 5 per sex per dose group
- **Study Design:**
  - ***Vehicle*****:** Citral was microcapsulated in a medium of 75% modified food-grade corn strarch and 25% sucrose.
  - ***Satellite groups and reasons they were added*****:** None
  - ***Clinical observations performed and frequency:***
    Animals were weighed at the start of the study, and at 4, 7, 11 and 14 days. Food

consumption was measured at 4, 7, 11 and 14 days. Clinical signs were recorded when animals were weighed.

- ***Heamatology and biochem.:*** Not examined
- ***Organs examined at necropsy****:*
  organ weight: right kidney, liver, spleen
  microscopic: lung, liver, kidney, spleen, nasal cavity, stomach, all gross lesions.

Results

- **NOAEL**
  Male & females: 570 mg/kg/day
- **LOAEL**
  Male & female: 1140 mg/kg/day (histopathological change in respiratory epithelium)

Remarks field for results:

- ***Body weight:****:* Reduction in body weight at day 4 of treatment period, and low body weight gain thereafter in both sexes at 1140 and 2280 mg/kg *(statisatic sig.),* and reduction in final body weight at 2280 mg/kg *(statisatic sig.).*
- ***Food/water consumption:*** Low food consumption at day 4 of treatment period in both sexes at 1140 and 2280 mg/kg and day 7 in both sexes at 2280 mg/kg *(statisic sig.)*
- ***Clinical signs*** *(description, severity, time of onset and duration)****:***
  Males & females*:* No clinical signs detected.
  Ophthalmologic findings: Not examined
- ***Mortality and time to death*****:** None
- ***Gross pathology incidence and severity*****:** None
- ***Organ weight changes***
  Males & females: Reduction in absolute weights of spleen, liver and kidney at 2280 mg/kg but no significant change in relative weight of these organs at any dose levels.
- ***Histopathology*** *(incidence and severity)* ***:***
  Nasal cavity: Minimal to mild hyperplasia and /or squamous metaplasia of the respiratory epithelium of the nose in both sexes at 1140 or 2280 mg/kg. These lesions were almost always located in anterior section of the nose and were present on the media side and ventral tips of the nasoturbinates. There was no inflammatory response associated with these changes.

Conclusions

Oral administration of citral in feed for 14 days caused reduction in body weights and lower body weight gain with low food consumption in both sexes at 1140 and 2280 mg/kg. Reduction in final body weights and in absolute weight of spleen, liver and kidney were noted in both sexes at 2280 mg/kg. Histopathological examination revealed the changes in nasal cavity. The minimal to mild hyperplasia and /or squamous metaplasia of the respiratory epithelium of the nose without inflammatory response occurred in both sexes at 1140 and 2280 mg/kg. A NOAEL was established at 570 mg/kg bw/day.

Data Quality

- **Reliabilities:** Valid with restriction because of no information of TG and GLP
- **Remarks field for Data Reliability :** Well conducted study, carried out by National Institute of Health, National Institute of Environmental Health Sciences, Research Triangle Park (USA).

Reference

Dieter M.P., et al., Food and Chemical Toxicol., 31,.7, pp.463-474, 1993

General Remarks

This study was conducted to examine the potential effects of microencapsulation on the toxicity of citral. Therefore, the toxicity by the feeding route was compared with that from bolus doses of the neat chemical in corn oil administered by gavage.

REPEATED DOSE TOXICITY (2)

Test substance

- Citral (CAS No 5392-40-5)
- Remarks: Source: CRARE, Lot No. 62938, purity: 98.20 %, mixture of two geometric isomers [54.99 % geranial (*trans*-isomer) and 43.60 % neral (*cis*-isomer) in citral received on June 18, 1996; 54.89 % geranial and 43.79 % in citral received on August 19, 1996], kept at ordinary temperature.

Method

- **Method/guideline:** OECD Preliminary Reproduction Toxicity Screening Test (TG 421)
- **Test type:** Preliminary Reproduction Toxicity Screening Test
- **GLP:** Yes
- **Year:** 1996
- **Species:** Rat
- **Strain:** Cij;CD (SD)
- **Route of administration:** Oral (by gavage)
- **Doses/concentration levels:** 0, 40, 200, 1,000 mg/kg/day (in corn oil)
- **Sex:** Male & Female
- **Exposure period:** Males; for 46 days
  Females; for 39-50 days from 14 days before mating to day 3 of lactation
- **Frequency of treatment:** Once a day
- **Control group and treatment:** Concurrent vehicle
- **Post exposure observation period:** None
- **Duration of test:** Male; for 46 days
  Female; for 39-50 days
- **Statistical methods:** Dunnett's test for continuous data and Mann-Whitney U test for quantal data

Test Conditions

– **Test Subjects:**
  · ***Age at study initiation*:** 10 week old for both sexes
  · ***Weight at study initiation*:** 315-391g for males, 207-254g for females
  · ***No. of animals per sex per dose*:** 12 per sex per dose group

– **Study Design:**
  · ***Vehicle*:** corn oil
  · ***Satellite groups and reasons they were added*:** none

- ***Clinical observations performed and frequency*:**
  General condition was observed once a day, body wt. and food consumption were determined at days 1(before dosing), 2, 5, 7, 10, 14 of treatment, thereafter once a week, but food consumption was not determined during mating period.
- ***Heamatology and biochem.:*** Not examined
- ***Organs examined at necropsy:***
  organ weight: testes, epididymis for males, ovaries for females
  microscopic: stomach, testes, epididymis for males, stomach, ovary, for females and liver for one female showed gross change in liver in the 1000mg/kg/day.

Results

- **NOAEL**
  Male & female: 200 mg/kg/day
- **LOAEL**
  Male & female: 1000 mg/kg/day (histopathological change in stomach)

Remarks field for results:

- ***Body weight:*** Low body weight gain during the treatment period in males at 1000 mg/kg *($p < 0.05$).*
- ***Food/water consumption:*** Low food consumption at day 5 of treatment period in both sexes *($p < 0.01$)* and day 4 of lactation in females *($p < 0.05$)* at 1000 mg/kg.
- ***Clinical signs** (description, severity, time of onset and duration)**:***
  Males: No clinical signs detected.
  Females: No clinical signs detected.
- ***Ophthalmologic findings:*** Not examined
- ***Mortality and time to death*:** None
- ***Gross pathology incidence and severity:*** Dilatation of renal pelvis (2/12), testis atrophy (1/12) and epididymis atrophy (1/12) in males, and dark red discoloration of liver (1/12) and partial thickening of mucosa of forestomach (10/12) in females at 1000 mg/kg.
- ***Organ weight changes:***
  Male: No statistic sig.
  Female: No statistic sig.
- ***Histopathology** (incidence and severity)**:***
  Male:
  Stomach: In forestomach, squamous hyperplasia (2/12) at 1000 mg/kg.
  Female:
  Stomach: In forestomach, squamous hyperplasia (10/12: *$p<0.01$*), ulcer (2/12), cellular infiltration of neutrophils (2/12) and granulation (6/12) in the lamina propria at 1000 mg/kg.

Conclusions

Daily oral administration of citral by gavage caused thickening of mucosal layer in the stomach in females at 1000 mg/kg, and histopathological examination in the forestomach revealed squamous hyperplasia in both sexes, ulcer, cellular infiltration of neutrophils and granulation in lamina propria in females at 1000 mg/kg. A NOAEL was established at 200 mg/kg/day.

Data Quality

- **Reliabilities:** Valid without restriction
- **Remarks field for Data Reliability:** Well conducted study, carried out by Safety Research Institute for Chemical Compounds Co.,Ltd. (Japan).

Reference

Ministry of Health, Labour and Welfare: Japan (2002) *Toxicity Testing Reports of Environmental Chemicals*, 9 in press.

General Remarks
This study was conducted to examine the reproductive/developmental toxicity as an OECD Preliminary Reproduction Toxicity Screening Test (TG 421). Hematological and blood chemical examinations were not performed. In addition, histopathological examination was not performed without stomach, testes and epididymis.

TOXICITY TO REPRODUCTION/DEVELOPMENT (1)

Test substance

- Citral (CAS No 5392-40-5)
- Remarks: Source: CRARE, Lot No. 62938, purity: 98.20 %, mixture of two geometric isomers [54.99 % geranial (*trans*-isomer) and 43.60 % neral (*cis*-isomer) in citral received on June 18, 1996; 54.89 % geranial and 43.79 % in citral received on August 19, 1996], kept at ordinary temperature.

Method

- **Method/guideline:** OECD: Preliminary Reproduction Toxicity Screening Test (TG 421)
- **Test type:** Preliminary Reproduction Toxicity Screening Test
- **GLP:** Yes
- **Year:** 1996
- **Species:** Rat
- **Strain:** Cij;CD (SD)
- **Route of administration:** oral (by gavage)
- **Doses/concentration levels:** 0, 40, 200, 1,000 mg/kg/day (in corn oil)
- **Sex:** Male & Female
- **Exposure period:** Males; for 46 days from 14 days before mating
  Females; for 39-50 days from 14 days before mating to day 3 of lactation
- **Frequency of treatment:** Once daily
- **Control group and treatment:** Concurrent vehicle
- **Post exposure observation period:** None
- **Duration of test:** Male; for 46 days
  Female; for 39-50 days
- **Statistical methods:** Dunnett's test for continuous data and Chi square test for quantal data

Test conditions

– **Test Subjects:**

· ***Age at study initiation*:** 10 week old for both sexes
· ***Weight at study initiation*:** 315-391 g for males, 207-254 g for females
· ***No. of animals per sex per dose*:** 12 per sex per dose group

– **Study Design:**
The animals were sacrificed on the day 4 of lactation for females. Females with no delivery were killed on the day 26 of pregnancy.

- ***Vehicle*:** corn oil
- ***Satellite groups and reasons they were added*:** none
- ***Mating procedures*:** Male/female per cage; 1/1, length of cohabitation; for 14 days, until proof of pregnancy (sperm detection in vagina)
- ***Clinical observations performed and frequency*:**

  Parent: General condition was observed once a day, body wt. and food consumption were determined at days 1(before dosing), 2, 5, 7, 10, 14 of treatment, thereafter once a week.

  Fetus: General appearance once a day after birth

  Hematology, biochemistry and urinalysis : Not examined
- ***Organs examined at necropsy*:**

  Parent: organ weight: testes, epididymis for males, ovaries for females

  microscopic: stomach, testes, epididymis for males, stomach, ovary, for females and liver for one female showed gross change in liver in the 1000 mg/kg/day.

  Fetus: full macroscopic examinations on all of pups
- ***Parameters assessed during study*:**

  Body wt. (once a week), food/water consumption (once a week), No. of pairs with successful copulation, copulation index (No. of pairs with successful copulation/No. of pairs mated x 100), pairing days until copulation, No. of pregnant females, fertility index = (No. of pregnant animals/No. of pairs with successful copulation x 100), No. of corpora lutea, No. of implantation sites, implantation index (No. of implantation sites/No. of corpora lutea x 100), No. of living pregnant females, No. of pregnant females with parturition, gestation length, No. of pregnant females with live pups on day 0, gestation index (No. of females with live pups/No. of living pregnant females x 100), No. of pregnant females with live pups on day 4, delivery index (No. of pups born/No. of implantation sites x 100), No. of pups alive on day 0 of lactation, live birth index (No. of live pups on day 0/No. of pups born x 100), sex ratio (Total No. of male pups/Total No. of female pups), No. of pups alive on day 4 of lactation, viability index (No. of live pups on day 4/No. of live pups on day 0 x 100), body wt. of live pups (on day 0 and 4)

Results

- **NOAEL and LOAEL for reproductive toxicity:**

  NOAEL: 1000 mg/kg/day

  LOAEL: more than 1000 mg/kg/day
- **NOAEL and LOAEL for developmental toxicity:**

  NOAEL: 200 mg/kg/day

  LOAEL: 1000 mg/kg/day

Parental data with dose level (with NOAEL value):

Partial thickening of mucosal layer in the stomach in autopsy in females and squamous hyperplasia, ulcer and granulation in lamina propria in histopathological examination in both sexes were observed at 1,000 mg/kg. However, as for parental toxicity, no statistically significant effects were detected in reproductive ability, organ weights or histopathology of the reproductive organs of both sexes, delivery or maternal behavior.

- **Foetal data with dose level (with NOAEL value):**

At 1,000 mg/kg, statistically significant decreases in body weight of male and female pups was observed during lactation period ($p<0.01$). No effects were revealed in viability, general appearance or autopsy of pups.

Dose level (mg/kg/day)		**0**	**40**	**200**	**1,000**
No. of pairs mated		12	12	12	12
No. of pregnant females		12	9	12	10
No. of pups born (Mean ± SD)		13.7 ±2.0	14.0 ±2.5	14.1 ± 2.9	13.1 ± 1.6
No. of implantation sites (Mean ± SD)		15.6 ±1.9	15.1 ±2.7	15.2 ±2.4	15.2 ±0.8
No. of pups alive on day 0 of lactation (Mean ± SD)			12.5 ±3.7	13.4 ±3.0	14.1 ±2.9	12.5 ±2.3
No. of pups alive on day 4 of lactation (Mean ± SD)			12.3 ±4.2	13.3 ±2.8	13.8 ±3.0	12.1 ±2.8
Body weight of live pups (%) (Mean ± SD)						
on day 0	**Males**		6.81 ± 0.48	6.62 ±0.64	6.43 ±0.82	5.88 ±0.56**
	Females		6.33 ±0.61	6.34 ±0.63	6.04 ±0.80	5.34±0.70**
on day 1	**Males**		7.52± 0.58	7.50 ±0.71	7.08 ±1.11	6.31 ±0.77**
	Females		7.09 ±0.71	7.07 ±0.69	6.61 ±1.13	5.69 ±0.97**
on day 4	**Males**		10.77±0.80	10.88 ±1.34	10.24 ±1.87	8.77 ±0.84**
	Females		10.08 ±0.99	10.32 ±1.50	9.71 ±1.81	7.98 ±1.15**

**p<0.01

Remarks field for results:

- ***Mortality and day of death*:** None
- ***Body weight*:** Low body weight gain during the treatment period in males at 1000 mg/kg *(p< 0.05)*.
- ***Food/water consumption:*** Low food consumption on day 5 of treatment period (male and female*: p<0.05*) and on day 4 of lactation period (female: *p<0.01*).
- ***Reproductive data*:** No statistically significant differences from controls.
- ***Fetal data*:** Although there were no statistically significant differences from controls in number of pups and viability, significantly low body weights of pups in both sexes during lactation period were noted at 1000 mg/kg (p<0.01).
- ***Grossly visible abnormalities, external, soft tissue and skeletal abnormalities*:** no statistically significant effects

Conclusions

No effects of citral were detected in reproductive parameters of parents received 1,000 mg/kg by oral gavage. However, significantly low body weights in live pups of 1,000 mg/kg group were detected on day 0 and did not recover until day 4 of lactation period. Therefore, the NOAELs for reproductive and developmental toxicity were established at 1,000 mg/kg/day and 200 mg/kg/day, respectively.

Data quality

- **Reliabilities:** Valid without restriction
- **Remark s field for Data Reliability:** Well conducted study, carried out by Safety Research Institute for Chemical Compounds Co., Ltd. (Japan).

Reference

Ministry of Health, Labour and Welfare: Japan (2002) *Toxicity Testing Reports of Environmental Chemicals*, 9 in press.

General Remarks

TOXICITY TO REPRODUCTION/DEVELOPMENT (2)

Test substance

- Citral (CAS No 5392-40-5)
- Remarks: Source: Citrus and Allied Essences Ltd (Flora Park, NY, USA), Lot No. 9966, Purity: approx. 90 % (isomeric distribution of approx. 35% neral and 55% geranial, impurity: unknown)

Method

- **Method/guideline:** Unknown
- **Test type:** Mammalian developmental toxicity by inhalation
- **GLP:** No data
- **Year:** 1991
- **Species:** Rat
- **Strain:** (COBS) CD BR (SD)
- **Route of administration:** inhalation
- **Doses/concentration levels:** 0, 10, 34 ppm as vapour, or 68 ppm as an aerosol/vapour mixture
- **Sex:** Female
- **Exposure period:** For 9 days from day 6 to 15 of pregnancy
- **Frequency of treatment:** 6 hr/day, daily
- **Control group and treatment:** Concurrent vehicle
- **Post exposure observation period:** For 5 days
- **Duration of test:** For 20 days
- **Statistical methods:** Dunnett's test, chi-square test and/or Fisher's exact probability test

Test conditions

– **Test Subjects:**

· ***Age at study initiation*:** 9-11 weeks old for both sexes
· ***Weight at study initiation*:** No data
· ***No. of animals per sex per dose*:** 25 females per dose group

– **Study Design:**

The pregnant females were sacrificed on day 20 of gestation, and the foetuses were removed and evaluated for gross, visceral and skeletal malformation.

· ***Vehicle*:**
· ***Satellite groups and reasons they were added*:** None
· ***Mating procedures*:** Male/female per cage; 1/2, length of cohabitation; at the most 4

days, until proof of pregnancy (formation of vaginal closing or sperm detection in vagina)

- ***Clinical observations performed and frequency*:**
  Female: General appearance twice a day
  Hematology, biochemistry and urinalysis: Not examined
- ***Organs examined at necropsy*:**
  Parent: organ weight: Not examined
  microscopic: Not examined
  Foetal: full macroscopic examinations on all of foetuses
- ***Parameters assessed during study*:**
  Female: Body wt. (recorded on gestation days 0, 2, 4, 6, 8, 12, 16 and 20), No. of corpora lutea, No. of implantation sites, pre-implantation loss, post-implantation loss, and resorption.
  Foetus: Body weight, autopsy and sex for all foetus, visceral examination for one-half of the foetuses in each litter and skeletal examination for the remaining foetuses.

Results

- **NOAEL**
  Female: 34 ppm for 6 hr/day
  Foetus: 68 ppm for 6 hr/day
- **LOAEL**
  Female: 68 ppm for 6 hr/day
  Foetus: more than 68 ppm for 6 hr/day

- **Actual dose received by dose level by sex if available:**
  0, 10, 34, 68 ppm for 6 hr/day

- **Maternal data with dose level (with NOAEL value):**
  One female aborted on day 10 and one female was killed in a moribund condition on day 17 of gestation in 68 ppm group. No statistically significant differences were noted in reproductive parameters between the control and the citral-exposed group.

Citra concentration(ppm)	0	10	34	68
No. of rats mated	25	25	25	25
No. of mortalities	0	0	0	2
No. of non-pregnants	1	4	2	3
No. of pregnants	24	21	23	20
No. of copora/dam	16.8 ± 1.8	16.3 ± 2.2	16.4 ± 2.7	16.7 ± 2.7
No. of implantations/dam	16.1 ± 3.2	15.1 ± 2.9	15.0 ± 4.8	14.8 ± 3.7
No. of pre-implantation loss(%)#	1.7 ± 5.5	3.7 ± 5.7	5.5 ± 11.4	4.3 ± 11.1
No. of post-implantation loss(%)#	2.5 ± 2.8	2.4 ± 2.1	2.2 ± 2.5	1.7 ± 2.3
No. of resorptions/dam	0.8 ± 0.9	0.7 ± 0.6	0.7 ± 0.8	0.6 ± 0.8
No. of live foetuses/litter	15.3 ± 3.2	14.5 ± 3.0	14.4 ± 4.8	14.2 ± 3.6
No. of dead foetuses/litter	0	0	0	0
Male/female sex ratio	0.91	1.05	0.96	0.86
Foetal body weight	3.9 ± 0.2	3.9 ± 0.3	4.0 ± 0.3	3.7 ± 0.6

#: Statistical analysis by arcsine transformation

- **Foetal data with dose level (with NOAEL value):**
  No statistically significant differences were noted in the incidence of malformation and variation for visceral and skeletal examinations between the control and the citral-exposured groups.

Remarks field for results:

- ***Chemical analysis:*** The low (10 ppm) and the medium (34 ppm) exposure levels were both vapour concentrations of citral, and the high (68 ppm) level was composed of 30.7 ppm citral aerosol with 37.0 ppm citral vapour. Aerosol particle size measurements in the high concentration chamber indicated an aerosol with a mass median aerodynamic diameter of 4.2 µm. It was estimated that approx. 90% of the aerosol mean was less than 10 µm aerodynamic diameter.

- ***Mortality and day of death*:** In 68 ppm group, one female aborted on day 10 and another one killed in a moribund condition on day 17 of gestation.
- ***Clinical signs:*** Ocular opacity, difficulty in breathing, nasal discharge, salivation, redness around the eyes, discoloured facial fur and rough hair coat in 68 ppm group.
- ***Body weight*:** Reduction in body weight during the exposure period and low body weights during days 8 to 20 of gestation in 68 ppm group *(Dunnett's test $p< 0.05$)*.
- ***Reproductive data*:** There was a slight increase in pre-implantation loss in the citral-exposed groups, but there was no significant reduction in litter size in the exposed groups.
- ***Fetal data*:** No statistically significant effects.
- ***Grossly visible abnormalities, external, soft tissue and skeletal abnormalities*:** No statistically significant effects. A total of 88 litters containing 1283 foetuses were examined. Gross observation revealed three foetal malformations including a right hind clubfoot in a control male, a short snout in a female and an umbilical hernia in a female from 68 ppm group. As for skeletal malformation and variation, malformations noted included missing or wavy ribs and scoliosis, with occurrences across all groups, including control. Poor ossification of the sternebrae was the most common skeletal developmental variation observed, occurring in approx. 70-80% of the litters in all groups. Alteration of the ribs was also frequently noted in all groups.

Conclusions

No significant maternal toxicity or adverse developmental effects in rats exposed to citral vapour at concentrations up to 34 ppm. Addition of aerosol component to produce a higher exposure concentration resulted in significant maternal toxicity. However, even in the presence of the maternal effects, no significant developmental toxicity was noted. A clear NOAEL was established at 34 ppm for pregnant female and 68 ppm for foetus.

Data quality

- **Reliabilities:** Valid with restriction because of no information of TG and GLP
- **Remarks field for Data Reliability:** Well conducted study, carried out by Lorillard Tobacco Co., Pathology Associates, Inc. and IIT Research Institute (USA)

Reference

Gaworski-CL, et al., Food and Chemical Toxicol., 30, 4, pp.269-275, 1992

General Remarks

GENETIC TOXICITY IN VITRO (BACTERIAL TEST) (1)

Test substance
- Citral (CAS No 5392-40-5)
- Remarks: Source: Sigma Chemical (St. Louis, MO, USA; a mixture of isomers, neral and geranial)

Method
- **Method/guideline**: D.M. Marson, B.N. Ames, Revised methods for Salmonella mutagenicity test, Mutat. Res., 113, 173-215(1983)
- **Test type** : Reverse mutation assay
- **GLP**: No data
- **Year**: 1997
- **Species/Strain:** *Salmonella typhimurium* TA102, TA100, TA98, TA97a
- **Metabolic activation:** With and without S9 from rat liver, induced with Aroclor1254
- **Statistical methods**: No data

Test conditions

– **Study Design:**

· ***Concentration*:** -S9: 300, 400, 500, 600, 700 µg/plate for TA100
200, 300, 400, 500, 600, 700 µg/plate for TA98 and 97a
5, 10, 25, 50, 100, 200, 300 µg/plate for TA102
+S9: 100, 200, 300, 400, 500, 600 µg/plate for TA100
300, 400, 500, 600, 700 µg/plate for TA98
200, 300, 400, 500, 600 700 µg/plate for TA97a
5, 10, 25, 50, 100, 200, 300, 400 µg/plate for TA102

· ***Number of replicates*:** 2
· ***Plates/test*:** 3
· ***Procedure*:** Pre-incubation
· ***Solvent*:** Ethanol
· ***Positive controls*:** SA for TA100/-S9, 2AA for TA100/+S9 and TA98/+S9, NPD for TA98/-S9, 4-NQNO for TA97a/-S9, 2AF for TA97a/+S9, MC for TA102/-S9, B-[a]-P for TA102/+S9

Results
- **Cytotoxic concentration:**
Toxicity was not observed up to 700 µg/plate in TA100, TA98 and TA97a, or 300 µg/plate in TA102 without S9 and 700 µg/plate in TA98 and TA97a, 600 µg/plate in TA100 or 400 µg/plate in TA102 with S9.

- **Genotoxic effects:**

	+	?	-
With metabolic activation:	**[ ]**	**[ ]**	**[X]**
Without metabolic activation:	**[ ]**	**[ ]**	**[X]**

Conclusions
Bacterial gene mutation is negative with and without metabolic activation

Data quality
- **Reliabilities:** Valid with restriction because of no information of TG
- **Remarks field for Data Reliability:** Well conducted study, carried out by Laboratorio de Toxicologia Ambiental, Escola Nacional de Saude Publica, Fundacao Oswaldo Cruz, Av. Brasil4365, Rio de Janeiro, and Depertmento de Biofisica e Biometria, Instituto de Biologia, Universidade de Estado do Rio de Janeiro, Rio de Janeiro.

Reference
Gomes-Carneiro M.R., et al., Mutation Research, .416, pp.129-136, 1998

General remarks

GENETIC TOXICITY IN VITRO (NON-BACTERIAL IN VITRO TEST) (1)

Test substance
- Citral (CAS No 5392-40-5)
- Remarks: Source: Japan Food Additives Association, purity: 98.2 %, kept in a refrigerator until use

Method
- **Method/guideline**: Unknown
- **Test type**: Chromosomal aberration test
- **GLP**: no data
- **Year**: 1979
- **Species/Strain:** CHL/IU cell
- **Metabolic activation:** Without
- **Statistical methods**: Unknown

Test conditions

– **Study Design:**

For continuous treatment, cells were treated for 24 or 48 hrs without S9.

- ***Concentration*:** -S9 (continuous treatment): 0.03 mg/ml (maximum dose, needed for 50% cell-growth inhibition)
- ***Plates/test*:** 2
- ***Solvent*:** DMSO
- ***Positive controls*:** Unknown

Results
- **Cytotoxic concentration:**

Toxicity was not observed up to 0.03 mg/ml in continuous treatment with or without S9 mix.

- **Genotoxic effects:**

	clastogenicity			polyploidy		
	+	?	-	+	?	-
– With metabolic activation:	**[ ]**	**[ ]**	**[X]**	**[ ]**	**[ ]**	**[X]**
– Without metabolic activation:	**[ ]**	**[ ]**	**[X]**	**[ ]**	**[ ]**	**[X]**

Remarks field for results

Structural chromosomal aberrations and polyploidy were not induced up to a maximum concentration of 0.03 mg/ml (50% cell-growth inhibition) on continuous treatment without an exogenous metabolic activation system.

Conclusion
Chromosomal aberration in CHL/IU cells is negative without metabolic activation.

Data quality

- **Reliabilities:** Valid with restriction because this study wasn't performed using test guideline and in accordance with GLP.
- **Remarks field for Data Reliability:** Well conducted study, carried out by Division of Mutageneses, Biologycal Safety Research center, National Research Institute of Hygienic Sciences (Japan).

Reference

Ishidate M, et al., Food and Chemical Toxicol., 22, ,8, pp.623-636, 1984

General remarks

This test was performed under a project operated by the Food Chemistry Division, Environmental Health Bureau, Ministry of Health and Welfare of Japan. This paper presented data from primary screening by the chromosomal aberration test on 242 food additives including citral. The result of citral was summarized, but the detailed information wasn’t reported in the paper because the result was negative.