Human Health Hazard Assessment

Diallyl Phthalate (DAP)
(CAS No. 131-17-9)

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INTRODUCTION

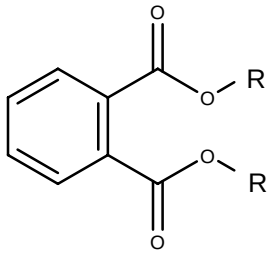
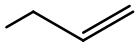
This review of diallyl phthalate (DAP) is a health hazard assessment only. For this assessment, an OECD SIDS Initial Assessment Report on DAP (OECD, 2004) was consulted. Information from this report was supplemented with relevant studies from more recent literature surveys conducted up to September 2006.

References not marked with an asterisk were examined for the purposes of this assessment. References not examined but quoted from the report as secondary citations are also noted in this assessment and marked with an asterisk.

Hazard information from this assessment is published also in the form of a phthalate hazard compendium providing a comparative analysis of key toxicity endpoints for 25 phthalates (NICNAS, 2007).

1. IDENTITY

1.1 Identification of the Substance

CAS Number:	131-17-9
Chemical Name:	1,2-Benzenedicarboxylic acid, di-2-propenyl ester
Common Name:	Diallyl phthalate (DAP)
Molecular Formula:	$C_{14}H_{14}O_4$
Structural Formula:	
	R = 
Molecular Weight:	246.27
Synonyms:	Allyl phthalate; Phthalic acid, diallyl ester o-Phthalic acid, diallyl ester
Purity/Impurities/Additives:	Purity ≥ 99% w/w

1.2 Physico-Chemical Properties

Table 1: Summary of physico-chemical properties

Property	***Value***
Physical state	Colourless, transparent liquid
Melting point	-70ºC
Boiling point	157ºC (0.67 kPa)
Density	1.12 (relative)
Vapour pressure	2.13×10^{-5} kPa (25 ºC)
Water solubility	0.148 g/L (20 ºC, pH 6.9–7.3)
Partition coefficient n-octanol/water (log Kow)	3.23 (20 ºC)

Henry's law constant	3.9×10^{-5} kPa-m³/mole (25 ºC)
Flash point	166 ºC (closed cup)

Source: OECD (2004)

2. USES

Production of diallyl phthalate (DAP) during 2002 was estimated at 4400 tonnes worldwide. Approximately half of the DAP produced is used as a monomer to form DAP prepolymer (i.e. semi polymerised polymers). DAP prepolymer is used for impregnated paper-decorated particleboard for wall materials or furniture, printing with UV-curable ink, grindstone, coil bobbin, and hot stamping foil. DAP is also used as a cross-linking agent in the manufacture of other polymers, such as polyvinyl chloride (PVC) and unsaturated polyesters. These polymers are used in finished products such as window frames, insulating varnish for coil and wire (OECD, 2004).

Diallyl phthalate is used as a plasticiser and carrier for adding catalysts and pigments to polyesters. It is used in electrical parts, laminating compounds (e.g. topcoat for fibreglass laminates), and impregnation of metal castings. Diallyl phthalate is also found in rubber compounds, epoxy formulations, and polyurethane foams (NTP, 1983).

In Australia, DAP is imported for use as a primary plasticiser for electrical insulation materials, flexible fibreglass topcoat laminates, adhesives and injection moulding materials. DAP is distributed also to various institutions and laboratories for analytical, pharmaceutical and biotechnological research.

3. HUMAN HEALTH HAZARD

3.1 Toxicokinetics

Previous Evaluations

Excretion, distribution and pharmacokinetic studies have been performed with rats (Fischer-344) and mice (B6C3F1) using ^{14}C-diallyl phthalate (DAP), labelled at the 2,3 position of the allyl alcohol moiety (Eigenberg, 1986*).

In excretion studies, ^{14}C-DAP was administered by gavage at 1, 10 or 100 mg/kg in rats and mice. Volatile metabolites, urine and faeces were collected for 24 hours. In rats, 25 – 30% of the ^{14}C-DAP dose was excreted as CO_2, and 50 – 70% appeared in urine within 24 hours. In mice, 6 – 12% of the dose was excreted as CO_2, and 80 – 90 % was excreted in the urine within 24 hours.

Tissue distribution and pharmacokinetic studies were conducted in rats and mice following intravenous administration (10 mg) of ^{14}C-DAP. DAP clearance from the blood was rapid in both rats and mice, with a half-life of approximately 2 minutes. No DAP was found in blood, liver, kidney, muscle, skin or small intestines 30 minutes after dosing.

Metabolites identified in urine (both species) included monoallyl phthalate, 3-hydroxypropylmercapturic acid and allyl alcohol.

Data not Reported in Previous Evaluations

No data.

Conclusion

DAP is extensively absorbed via the oral route and rapidly eliminated in rats and mice. Up to 30% of the dose is eliminated from lungs as CO_2, with the majority (50-90%) excreted in urine. A number of urinary metabolites have been identified, including 3-hydroxypropyl-mercapturic acid (HPMA) and allyl alcohol (AA). DAP does not accumulate in tissues.

3.2 Acute Toxicity

Previous Evaluations

Study	***Species***	***Results***
Oral	Rat (male)	LD_{50} 891 mg/kg bw
Oral	Rat (female)	LD_{50} 656 mg/kg bw
Oral	Mouse (male)	LD_{50} 1070 mg/kg bw
Oral	Mouse (female)	LD_{50} 1690 mg/kg bw
Dermal	Rabbit	LD_{50} 3300 mg/kg bw
Inhalation (1-h)	Rat (male)	LC_{50} 10.31 mg/L
Inhalation (1-h)	Rat (female)	LC_{50} 5.20 mg/L
Inhalation (4-h)	Rat (m & f)	LC_{100} 4.47 mg/L (100% mortality at Day 1)

Source: OECD (2004)

Data not Reported in Previous Evaluations

No data.

Conclusion

DAP exhibits low acute dermal and inhalation toxicity, with low to moderate acute oral toxicity in rodents.

3.3 Irritation

Skin Irritation

Previous Evaluations

Two reliable studies (DAISO, 1998*; Ethyl Corp., 1979*) showed little or no skin irritation potential for DAP in rabbits (occluded abraded and intact sites). There were no signs of toxicity or ill health in any rabbit during the observation period (24 and 72 hours after removal of the chemical).

Data not Reported in Previous Evaluations

No data

Conclusion

DAP caused minimal to no skin irritation in rabbits.

Eye Irritation

Previous Evaluations

DAP was found to be non-irritating to rabbit eyes in three studies, one of which is considered reliable (Ethyl Corp., 1979*). In this study, 0.1 ml of undiluted DAP was instilled into the right eye of 6 rabbits. The animals were examined after 1 and 4 hours and then daily on days 1, 2, 3, 4 and 7. Examination did not reveal any positive grades of redness or chemosis in any animals.

Data not Reported in Previous Evaluations

No data.

Conclusion

DAP did not cause eye irritation in rabbits.

3.4 Sensitisation

Previous Evaluations

One study, a mouse (CBA/CaBkl) local lymph node assay, was available for assessing the skin sensitisation potential of DAP. In this study, 25 µl of DAP solution (acetone/olive oil 4:1 vehicle) was applied to the ear surface of mice at concentrations of 0, 0.5, 5 and 50 % w/v on 3 consecutive days. There were no clinical signs of toxicity during the study. The Stimulation Index was reported as 3.23 at 5% w/v and 10.74 at 50 % w/v (DAP Consortium, 2003*). A Stimulation Index greater than 3.0 indicates a positive result.

Data not Reported in Previous Evaluations

No data.

Conclusion

DAP caused skin sensitisation in mice.

3.5 Repeated Dose Toxicity

Previous Evaluations

Five repeat dose oral toxicity studies were available for assessment with a 13-week NTP rat study (NTP, 1985*) considered as the key study, being well reported and conducted according to GLP. In this study, Fischer 344 rats (10 animals/sex/dose) were dosed by gavage with DAP at 0, 25, 50, 100, 200 and 400 mg/kg bw/day on 5 days/week for 13 weeks.

At 400 mg/kg bw/day, 8/10 male animals either died during the study or were killed due to a moribund condition. In this group, male body weight gain was decreased 12% relative to vehicle controls. Clinical signs, including diarrhoea, rough hair coat, alopecia, hunched

posture and general emaciation, were seen in both sexes at 400 mg/kg bw/day and less frequently at 200 mg/kg bw/day but not at lower doses.

At 400 mg/kg bw/day, gross abnormalities of the liver (enlargement, mottled and pale rough, granular or pitted surface) were observed in all 8 male animals that died early. In these animals, darkened or bright red lungs were also observed. Similar liver abnormalities were observed in the two surviving males and in most females at 400 mg/kg bw/day, and in 5/10 males at 200 mg/kg bw/day. The severity of lesions was dose related and greater in males than in females.

Histopathology indicated the liver to be the primary target organ, with periportal lesions (necrosis, fibrosis) of hepatic lobules and hepatocellular hyperplasia seen in both sexes at 200 and 400 mg/kg bw/day. Necrosis, fibrosis and biliary hyperplasia were not observed below 200 mg/kg bw/day. Hepatocellular alterations (hepatocellular basophilia, cellular and nuclear hypertrophy and hyperchromatism) in the periportal region were observed with decreasing frequency and severity at doses as low as 50 mg/kg bw/day in males and 100 mg/kg bw/day in females. Liver histopathology was not undertaken for the lowest dose group (25 mg/kg bw/day). Acute necrotizing colitis, characterised by loss of surface and glandular epithelium, varying degrees of mucosal and submucosal oedema, and acute inflammatory cell infiltration, was found in 7/8 male rats that died early at 400 mg/kg bw/day. In addition, 3 of these animals exhibited multifocal renal cortical tubular necrosis. Discolouration of kidneys was also observed in females at 400 mg/kg bw/day.

In this study a NOAEL of 50 mg/kg bw/day DAP for females was determined, with a LOAEL of 100 mg/kg bw/day for effects on the liver. The LOAEL for males was 50 mg/kg bw/day based on effects on the liver. A NOAEL for males could not be determined because no liver histopathology was undertaken for the low dose animals.

These NOAELs/LOAELs were consistent with results from another NTP study (14-day) in the same strain of rat. Doses (gavage) in this study ranged from 50 to 600 mg/kg bw/day (NTP, 1985*).

A NOAEL of 50 mg/kg bw/day was determined (in both sexes) in a 54-day rat (Sprague-Dawley) gavage study (DAP Consortium, 2004*) carried out at doses of 0, 16.7, 50 and 150 mg/kg bw/day. Effects at 150 mg/kg bw/day included periportal hepatocytes necrosis, enlargement and basophilia, bile duct proliferation and periportal fibrosis and stomach ulceration.

No DAP induced lesions (in any organ) were observed in a 14 day study using B6C3F1 mice, at dosage up to 600 mg/kg/day for up to 13 weeks. Deaths occurred in the 400 and 600 mg/kg/day groups, but not at lower doses (NTP, 1983*).

In a 13 week study, B6C3F1 mice were administered with DAP at doses of 0, 25, 50, 100, 200 and 400 mg/kg bw/day for 5 days/week. There was no statistically significant change in body weight gain and no DAP-related pathological alterations were observed (NTP, 1983*).

Data not Reported in Previous Evaluations

No data.

Conclusion

The liver appears to be the primary target organ. In rats, effects were seen in bile duct and periportal region of the liver. Effects were also seen in kidney and intestines. These effects were not seen in mice.

The repeated dose oral NOAEL in female rats was 50 mg/kg bw/day with a LOAEL of 100 mg/kg bw/day and the LOAEL for male rats was 50 mg/kg bw/day.

3.6 Genetic Toxicity

Previous Evaluations

In vitro studies

In bacterial reverse mutation studies, two key studies which were well-conducted under GLP were identified (MOL, Japan, 2000*; FMC Corp, 1986*). In these two studies DAP was tested for reverse mutation (mutagenicity) in *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538) both with and without metabolic activation, at concentrations of 1.22 - 5000 µg/plate and 25-6000 µg/plate.

Results indicated a weak positive response in strain TA100 (600 µg/plate and higher) without metabolic activation and negative with metabolic activation. All other strains showed negative responses in all cases. Cytotoxicity was observed from 1000 µg/plate (TA100) with and without exogenous metabolic activation but not the TA100 (FMC Corp, 1986*).

In a study using *Escherichia coli (*strain WP2uvrA/pKM10) at DAP concentrations of 1.22 to 5000 µg/plate both with and without exogenous metabolic activation, no cytotoxicity was shown over the concentration range tested. A weak positive response was observed at and above 1250 µg/plate with exogenous metabolic activation. No cytotoxicity was noted (MOL, Japan, 2000*).

Other bacterial mutagenicity tests have been reported as negative both with and without exogenous metabolic activation (Zeiger, 1985*, FMC Corp, 1977*).

In contrast to bacterial mutation assays, clear positive responses were observed in each of three mammalian cell assays, particularly in the presence of exogenous metabolic activation, where the toxic effects were reduced and higher dose levels could be achieved.

In mouse lymphoma cells (L5178Y) (Myhr, 1991*), DAP was mutagenic near 50-75 nl/ml (56-85 µg/ml, 46-49 % relative total growth) and showed dose-related increases up to 150 nl/ml (168 µg/ml, estimated from relative density = 1.12). At 150 nl/ml DAP induced 6-9 fold increases in mutation with or without metabolic activation. In another experiment, DAP induced chromosomal aberrations and an increase in sister chromatid exchange in Chinese hamster ovary (CHO) cells with exogenous metabolic activation at the highest dose tested (200-300 µg/ml) (Gulati et al, 1989*). DAP also induced micronucleus formation (at 20 µg/ml) in Chinese hamster lung (CHL/IU) cells, with exogenous metabolic activation (MOL, Japan, 2002*).

In vivo studies

In a sex-linked recessive lethal (reciprocal translocation) (SLRL) test (Valencia, 1985*), *Drosophila melanogaster* were treated with feed containing DAP at doses of 0, 100 and 140 ppm or injected with 500 ppm of the chemical 24 hours prior to mating with untreated females. Results were negative from both routes of DAP administration.

In a mouse micronucleus assay (Shelby, 1993*) animals were injected intraperitoneally with DAP at 0, 43.8, 87.5 and 175 mg/kg bw. There were no statistically significant increases in the frequency of micronucleated polychromatic erythrocytes (PCE) in any test group when compared to controls.

In a chromosome aberration test (Shelby, 1995*), duplicate groups of male mice were injected intraperitoneally with DAP at 0, 75, 150 and 300 mg/kg bw). In the first group there was a small but statistically significant increase in the number of chromosomal aberrations in bone marrow cells at the high dose (7.5±1.18%) when compared to the concurrent vehicle control (3.25±1.25%), whereas the second group produced negative results.

Data not Reported in Previous Evaluations

DAP was negative (prophage induction in *E. coli B/WP2*) in the Microscreen assay, both with and without exogenous metabolic activation (Rossman et al, 1991). DAP was also negative in the *Salmonella* umu test, both with and without exogenous metabolic activation, which measures induction of the *umuDC-lacZ* gene in *Salmonella typhimurium* TA 1535 (Yasunaga et al, 2004).

Conclusion

In *in vitro* studies, DAP was weakly mutagenic in one strain of bacteria *(*strain WP2uvrA/pKM10) in the presence of metabolic activation but was negative in majority bacterial with and without metabolic activation. A clear positive response was observed in a mouse lymphoma assay both with and without metabolic activation. DAP induced chromosomal aberrations in CHO cells both with and without metabolic activation and sister chromatid exchanges in CHO cells with metabolic activation only. In addition, DAP induced micronucleus formation in CHL/IU cells with exogenous metabolic activation.

In *in vivo* studies, DAP was not mutagenic in either a mouse micronucleus or SLRL test (*Drosophila melanogaster).* Results on DAP induced chromosome aberrations in mouse bone marrow cells were equivocal as effects were only seen at the highest dose in 1 of 2 groups. The result of this study conflicts with the micronucleus study reported by the same author although both study types investigate similar endpoints.

Overall, whilst DAP is mutagenic *in vitro* and generally negative results seen in *in vivo* studies, it is not possible to conclude the genotoxic potential of this phthalate.

3.7 Carcinogenicity

Previous Evaluations

In an NTP study (NTP, 1985*), Fischer 344 rats (50 animals/group/sex) were administered DAP (by gavage) at 0, 50 and 100 mg/kg bw/day (5 days/week) for 103 weeks. Survival rates and mean body weights of DAP-treated animals were similar to controls.

DAP administration produced a dose-dependent increase in chronic liver injury. Male and female animals dosed at 100 mg/kg bw/day developed chronic liver disease characterised by periportal fibrosis and accumulation of pigment, and severe bile duct hyperplasia, with pigment accumulation also occurring at the 50 mg/kg bw/day dose in both sexes.

An increased incidence of mononuclear cell leukaemia (MCL) was also seen in female animals at both test doses, which was significantly greater than controls ($p<0.05$ by trend tests) in the 100 mg/kg bw/day dose group (vehicle control (15/50, 30%); low dose (15/43, 35%) and high dose (25/49, 51%). No increase in MCL was observed in male animals.

In another NTP study (NTP, 1983*), B6C3F1 mice (50 animals/group/sex) were administered (by gavage) DAP at doses of 0, 150 and 300 mg/kg bw/day (5 days/week) for 103 weeks. Survival rates and mean body weights of DAP-treated animals were similar to controls. A dose related increase in the development of chronic inflammation and hyperplasia of the forestomach were observed and were considered related to DAP treatment but the data were insufficient to indicate a clear cause and effect.

Data not Reported in Previous Evaluations

No data.

Conclusion

In chronic studies (103 weeks) in both mice and rats, evidence of DAP induced carcinogenicity was equivocal. A dose-related increase in forestomach hyperplasia and squamous cell papillomas was considered to be treatment related but the data were insufficient to indicate a clear cause and effect. An increase in mononuclear cell leukaemia (only tumour type reported), seen in female rats only, was statistically significant but this tumour type is well known to occur spontaneously with high incidence in the rat strain tested (F344).

3.8 Reproductive Toxicity

Previous Evaluations
In a single combined reproduction/developmental toxicity screening test, DAP was administered (by gavage) to Sprague-Dawley rats (10 animals/dose/sex) at 0, 16.7, 50 and 150 mg/kg bw/day from 14 days prior to mating to 4 days post-partum (maximum 54 days) (DAP Consortium, 2004*).

At 150 mg/kg bw/day, 3 female died in extremis, with clinical signs associated with possible dystocia, considered to be due to the marked hepatotoxicity seen in these animals. No mortalities occurred in other test groups. No further details were provided on the diagnosis of the dystocia and it was considered most likely to be associated with the severe maternal toxicity seen at this dose. Statistically significant increases in histopathological findings in the liver (periportal hepatocyte necrosis, enlargement and basophilia, bile duct proliferation and periportal fibrosis) were seen in high dose (150 mg/kg bw/day) animals only. One of the female rats that died in extremis had ulceration of the glandular region of the stomach.

There were no treatment-related effects seen on the fertility of either male or female animals, as shown by the high pregnancy rate in all DAP-treated groups and the lack of significant differences in the distribution of pre-coital intervals for all dose groups. No effects were observed on numbers of corpora lutea, implantations or litter size, although effects on new-borns and live new-borns were not evaluated in the three rats that dies or were killed. There were no treatment-related effects on offspring viability, growth or development from conception to early lactation. No macroscopic abnormalities were seen in offspring.

A NOAEL of 50 mg/kg bw/day was established for maternal toxicity with a LOAEL of 150 mg/kg bw/day due to death and liver effects. The NOAEL for reproductive toxicity was 50 mg/kg bw/day.

Data not Reported in Previous Evaluations

No data.

3.8.1 Mode of Action

Data not Reported in Previous Evaluations

In an *in vitro* oestrogen receptor binding assay for a number of dialkyl phthalates, diallyl phthalate exhibited a relatively high binding affinity for the oestrogen receptor, but was much less potent (several orders of magnitude) than 17β-estradiol (Nakai et al, 1999).

Conclusion

Effects on fertility

There were no treatment-related effects seen on fertility in male or female rats in any test group, as evidenced by the high pregnancy rate and lack of histopathological changes seen in the reproductive organs of parental animals. Dystocia occurred at the highest doses and was considered most likely to be associated with the severe maternal toxicity seen at this dose. The NOAEL for reproductive toxicity was 50 mg/kg bw/day.

Developmental effects

Apart from mortalities in 3/10 females in the high dose group (effects on new-borns and live new-borns were not evaluated in the three rats that dies or were killed), there were no treatment-related effects seen on development in male or female rats in any test group, as evidenced by a lack of effects on offspring viability, growth and development from conception to early lactation. No macroscopic abnormalities were seen in offspring.

4. HAZARD CHARACTERISATION

DAP is extensively absorbed and rapidly eliminated (following ingestion) in rats and mice. The majority (50-90% dose) is excreted in urine. A number of urinary metabolites have been identified, including monoallyl phthalate, 3-hydroxypropylmercapturic acid (HPMA) and allyl alcohol (AA).

DAP exhibits low acute dermal and inhalation toxicity, with low to moderate acute oral toxicity in rodents.

DAP did not produce either eye or skin irritation in rabbits, but produced a positive response in the mouse local lymph node assay for skin sensitisation.

In repeat dose studies (13-week and 103-week) in F344 rats, the liver was the primary target organ, with pre-neoplastic effects seen in bile duct and periportal regions. Effects were also seen in kidney (cortical tubular necrosis) and intestines (acute necrotizing colitis) in the 13-week study. These effects were not seen in studies of similar dose and duration in mice. A repeated dose oral NOAEL of 50 mg/kg bw/day was determined for female rats in both sub-chronic and chronic studies. No repeat dose dermal or inhalation studies were available for assessment.

In vitro, DAP was weakly mutagenic in two strains of bacteria with and without metabolic activation but negative in most strains tested. Positive responses were reported in a mouse lymphoma (mutation) assay and a chromosomal aberration study in CHO cells, both with and without metabolic activation. DAP induced sister chromatid exchanges in CHO cells and micronucleus formation in CHL/IU cells in the presence of metabolic activation. *In vivo*, DAP was not mutagenic in either a mouse micronucleus or *Drosophila melanogaster* SLRL test. Results on DAP induced chromosome aberrations in mouse bone marrow cells were equivocal. Overall, whilst DAP is mutagenic *in vitro* and generally negative results seen in *in vivo* studies, it is not possible to conclude the genotoxic potential of this phthalate.

In chronic studies (103 weeks) in both mice and rats, evidence of DAP induced mononuclear cell leukaemia (MCL) seen in female rats. However, as MCL is a common neoplasm in Fischer 344 rats and its increased incidence after chronic exposure to some substances is considered to be a strain specific effect (Caldwell 1999). Caldwell (1999) notes that MCL has not been found in other mammalian species and has no histologically comparable tumour type in humans. This neoplasm observed in rats is unlikely to be relevant to humans.

The development of chronic inflammation, hyperplasia and papillomas of the forestomach in B6C3F1 mice were considered related to DAP treatment but the data were insufficient to indicate a clear cause and effect (NTP, 1983*). In addition, this effect may not be relevant for humans, due to the fact that humans do not have a forestomach and the comparative residence time for ingested substances in the human stomach is much lower. It was also reported that a maximally tolerated dose (MTD) for the purposes of carcinogenicity testing might not have been achieved in this study.

The presence of allyl alcohol (AA) as one of the metabolites of DAP, has led to the hypothesis that the liver effects seen with DAP was treatment-related, as AA is a known potent periportal hepatotoxicants (OECD, 2004). For other phthalates (e.g. DEHP and

DINP), liver effects are associated with peroxisome proliferation, however no data were available on potential DAP-induced peroxisome proliferation.

Overall, it was considered that the tumour types seen in animal studies are of limited significance to humans, but the non-neoplastic effects (liver) seen in rats may be relevant.

In a single, combined reproduction/developmental toxicity screening test, DAP was administered (by gavage) to rats (14 days prior to mating to 4 days post-partum). Liver effects, consistent with those seen in sub-chronic and chronic studies were seen in high dose group (150 mg/kg bw/day). There were no treatment-related effects seen on standard reproductive parameters. The NOAEL for maternal toxicity was considered as 50 mg/kg bw/day due to maternal death at 150 mg/kg bw/day. The NOAEL for reproductive toxicity was 50 mg/kg bw/day.

5. HUMAN HEALTH HAZARD SUMMARY TABLE

Phthalate	***Acute Toxicity***	***Irritation & Sensitisation***	***Repeated Dose Toxicity***	***Genetic Toxicity***	***Carcinogenicity***	***Fertility***	***Developmental Toxicity***
Diallyl phthalate (DAP)	Oral Rat: LD50 = 656-891 mg/kg bw. Dermal Rabbit: LD50 = 3300 mg/kg bw Inhalation Rat (1 hr): LC50 = 5.20-10.31 mg/L	Skin Irritation: negative Eye Irritation: negative Skin sensitisation: positive (LLNA)	Rat: NOAEL = 50 mg/kg bw/d (f) LOAEL = 100mg/kg bw/d; hepatocellular hypertrophy/renal tubular necrosis (f)	<u>*In vitro*</u> Negative in bacterial mutation assays Negative in bacterial microscreen assay Positive in chromosome aberrations, sister chromatid exchange and micronuclei assays Positive in mouse lymphoma assay <u>*In vivo*</u> Equivocal in chromosome aberrations assay Negative in mouse micronucleus tests Negative in sex-linked recessive lethal assays	*Two year dietary study* F344 rat: NOAEL = not established LOAEL = 100 mg/kg bw/d; ↑ mononuclear cell leukaemia (MCL) (f). *Two year dietary study* B6C3F1 mouse: ↑ forestomach papillomas (equivocal causality). .	*One generation study* Rat: NOAEL= 50 mg/kg bw/d LOAEL = 150 mg/kg bw/d	Insufficient data

f: female; ↑: increase.

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