Tonalide: Human health tier II assessment

08 March 2019

Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
Ethanone, 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)-	1506-02-1
Ethanone, 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)-	21145-77-7

Preface

This assessment was carried out by staff of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) using the Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework.

The IMAP framework addresses the human health and environmental impacts of previously unassessed industrial chemicals listed on the Australian Inventory of Chemical Substances (the Inventory).

The framework was developed with significant input from stakeholders and provides a more rapid, flexible and transparent approach for the assessment of chemicals listed on the Inventory.

Stage One of the implementation of this framework, which lasted four years from 1 July 2012, examined 3000 chemicals meeting characteristics identified by stakeholders as needing priority assessment. This included chemicals for which NICNAS already held exposure information, chemicals identified as a concern or for which regulatory action had been taken overseas, and chemicals detected in international studies analysing chemicals present in babies' umbilical cord blood.

Stage Two of IMAP began in July 2016. We are continuing to assess chemicals on the Inventory, including chemicals identified as a concern for which action has been taken overseas and chemicals that can be rapidly identified and assessed by using Stage One information. We are also continuing to publish information for chemicals on the Inventory that pose a low risk to human health or the environment or both. This work provides efficiencies and enables us to identify higher risk chemicals requiring assessment.

The IMAP framework is a science and risk-based model designed to align the assessment effort with the human health and environmental impacts of chemicals. It has three tiers of assessment, with the assessment effort increasing with each tier. The Tier I assessment is a high throughput approach using tabulated electronic data. The Tier II assessment is an evaluation of risk on a substance-by-substance or chemical category-by-category basis. Tier III assessments are conducted to address specific concerns that could not be resolved during the Tier II assessment.

These assessments are carried out by staff employed by the Australian Government Department of Health and the Australian Government Department of the Environment and Energy. The human health and environment risk assessments are conducted and published separately, using information available at the time, and may be undertaken at different tiers.

This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

For more detail on this program please visit:**www.nicnas.gov.au**

Disclaimer

ACRONYMS & ABBREVIATIONS

Grouping Rationale

The chemical, ethanone, 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)-, (tonalide) has been assigned two CAS Nos. by the Chemical Abstracts Service (21145-77-7 and 1506-02-1). These two CAS Nos. are numerical identifiers for the same chemical substance and both are listed on the AICS. This assessment provides findings that are applicable to both CAS Nos.

The stereochemistry of tonalide is not specified by the chemical name. The technical form of tonalide used industrially is a racemic (equal) mixture of the two optical stereoisomers of this chemical (EU RAR, 2008).

Import, Manufacture and Use

Australian

The chemicals have reported cosmetic use in hand and body wash products.

International

The following international uses were identified through Galleria Chemica; the European Union (EU) Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) dossiers; Substances and Preparations in the Nordic countries (SPIN) database; the United States (US) Environmental Protection Agency (EPA) Chemical and Product Categories (CPCat); the US Personal Care Product Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary and Cosmetic Ingredients; Cosmetic Ingredients and Substances (CosIng) database; and European Union Risk Assessment Report (EU RAR, 2008).

The chemicals have reported cosmetic uses as fragrance ingredients in perfumes and personal care products. The chemicals are listed on the IFRA transparency list of fragrance materials (IFRA, 2017).

The chemicals have reported domestic uses in:

- washing and cleaning products;
- air care products; and
- car care products.

The chemicals have reported commercial uses in surface treatments, absorbents and adsorbents.

Restrictions

Australian

No known restrictions have been identified.

International

Using tonalide in cosmetics in the European Union is subject to the restrictions described in Annex III of Regulation 1223/2009/EC (Cosmetics Regulation) (Ref no 182). Tonalide may be used in cosmetics and personal care products at a maximum concentration of 0.1 % in leave-on products (except hydroalcoholic products), 1 % in leave-on hydroalcoholic products, 2.5 % in fine fragrance, 0.5 % in fragrance cream and 0.2 % in rinse-off products (CosIng).

Existing Worker Health and Safety Controls

Hazard Classification

Tonalide is not listed on the Hazardous Chemical Information System (HCIS) (Safe Work Australia).

Exposure Standards

Australian

No specific exposure standards are available.

International

No specific exposure standards are available.

Health Hazard Information

Toxicokinetics

The available data suggest that complete absorption after oral or dermal exposure is unlikely and no toxicokinetic data are available for inhalation exposure (EU RAR, 2008).

In rats, dermal absorption was estimated to be approximately 19 %. Studies in human volunteers (n=3) and in vitro studies indicated that absorption in humans is lower (up to 4.1 %) (EU RAR, 2008).

Tonalide shows a rapid and widespread distribution without any evidence of accumulation. Tonalide was found in rat milk at 9.43 µg/L milk after 14 days of oral administration (20 mg/kg bw/day) (EU RAR, 2008).

After oral and intravenous administration, numerous metabolites were detected in urine, faeces and liver samples; however, none of the metabolites were characterised. The major excretion route in rats is via the faeces, while it is the urine in humans and pigs (EU RAR, 2008).

An environmental assessment determined that tonalide is persistent and toxic to the environment; however, it was not considered bioaccumulative (NICNAS).

Biomonitoring

Tonalide has been detected in human breast milk, adipose tissue and blood.

Several studies measuring tonalide in human milk are available. Reported concentrations of tonalide in whole milk ranged between 0.59–53 µg/kg (EU RAR, 2008).

The concentrations of tonalide in adipose tissue varied between 1–72 µg/kg fat (EU RAR, 2008). Tonalide was detected in all of the 29 tested samples.

Tonalide have been detected at concentrations up to 247 ng/L in human blood (EU RAR, 2008).

Acute Toxicity

Oral

Tonalide is expected to have moderate acute toxicity via the oral route and warrant hazard classification (see **Recommendation** section). The reported median lethal doses (LD50) are between 570–1377 mg/kg bw.

In two acute toxicity studies conducted similarly to the Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 401, Wistar rats (5/sex/dose) were orally treated (gavage) with single doses of 700, 840, 1008, 1209, 1451 mg/kg bw or 1050, 1260, 1512, 1815 or 2177 in mg/kg bw of tonalide in isopropyl myristate (w/v) and observed for 14 days. Mortalities occurred at all doses. Clinical signs of toxicity included sluggishness, piloerection and haematuria. LD50 values of 920 and 1150 mg/kg bw were reported (EU RAR, 2008; REACH).

Similar findings were reported in two non-guideline studies in female Sprague Dawley (SD) rats (5/dose) treated with single doses of 1260, 1588, 2000 or 2520 mg/kg bw or 215, 464, 1000 or 2150 mg/kg bw of tonalide in ethanol/polyethylene glycol or ethanol (w/v). Animals were observed for 14 days or 7 days, respectively. LD50 values of 1150 and 1377 mg/kg bw were reported (EU RAR, 2008; REACH).

Other reported LD50 values from non-guideline studies in rats include 825 mg/kg bw and 570 mg/kg bw (EU RAR, 2008; REACH).

Dermal

Tonalide is expected to have low acute toxicity via the dermal route. The reported LD50 is >5000 mg/kg bw.

In a dermal acute toxicity study conducted similarly to OECD TG 402, male SD rats (5/dose) were treated with a single application of 464, 1000, 2150 (in 10 % ethanol), 4640 (in 20 % ethanol), or 10000 (in 40 % ethanol) mg/kg bw of tonalide onto shaved skin. One mortality occurred at each of 1000 and 4640 mg/kg bw and 5 mortalities occurred at 10000 mg/kg bw. Clinical signs of toxicity were mainly seen at the highest dose and included depression, hunching, hind limb weakness and prostration. An LD50 of 7940 mg/kg bw was reported (EU RAR, 2008; REACH).

In a pre-guideline dermal acute toxicity in rabbits, dermal administration of a single dose of 5000 mg/kg bw did not result in mortality within the 14-day observation period. Slight signs of skin irritation were observed in all animals. The LD50 was >5000 mg/kg bw (EU RAR, 2008; REACH).

Inhalation

No data are available.

Corrosion / Irritation

Skin Irritation

Tonalide may be slightly irritating to skin particularly following repeated exposure (see ***Repeated dose toxicity*** section). The effects are not sufficient to warrant hazard classification.

Animal studies indicate that tonalide may be slightly more irritating after irradiation with UV light; however, these effects have not been reproduced in humans (see ***Observation in humans*** section) and an in vitro guideline study was negative for phototoxicity.

In a study conducted according to directive 79/831/EEC, 3 female New Zealand White (NZW) rabbits received topical applications of tonalide (0.5g in 0.5 mL water) under a 6.25 cm^2 semi-occlusive patch for 4 h on clipped dorsal skin and were observed 1, 24, 48, 72 h and 7 days after patch removal. No erythema or oedema was seen in any rabbit at any of the time points (EU RAR, 2008; REACH).

In a study conducted according to directive 79/831/EEC, 6 female NZW rabbits received topical applications of 0.5 mL of tonalide (50 % in diethyl phthalate) under a 6.25 cm^2 semi-occlusive patch for 4 h on clipped dorsal skin and were observed 1, 24, 48, 72 h and 7 days after patch removal. The irritation scores at the 3 different time-points (24, 48 and 72 h) were combined and averaged. The average irritation scores for the 6 individual rabbits were 0, 0, 1, 2, 0, 0.33 for erythema and 0, 0, 0.67, 1.33, 0, 0 for oedema (EU RAR, 2008; REACH).

Several in vivo non-guideline photoirritation studies with limited reported information have been conducted. In five guinea pig photoirritation studies, dermal reactions were observed at concentrations between 0.3–50 % of tonalide in ethanol up to 72 h after irradiation. In rabbits, positive photoirritation reactions were reported at concentrations between 1–20 % of tonalide in ethanol (EU RAR, 2008).

In a phototoxicity assay conducted according to OECD TG 432 (neutral red uptake phototoxicity assay), mouse fibroblast cells were exposed to 50 µL of tonalide in Hank's Balanced Salt Solution (HBSS) containing 0.5 % ethanol at concentrations of 0.992–56.2 µg/mL for 1 h followed by irradiation for 50 min at a total irradiation dose of 5 J/cm^2. The mean photo effect (MPE) and photo- irritation factor (PIF) were below the threshold values for tonalide to be considered a photoirritant (<0.1 and <5.0, respectively). Therefore, tonalide was not considered to be a photoirritant (EU RAR, 2008).

Eye Irritation

Tonalide may be slightly irritating to eyes. The effects are not sufficient to warrant hazard classification.

In an OECD TG 405 eye irritation study, 0.1 g of tonalide was applied to one eye of 3 male NZW rabbits while the other eye served as the control. The irritation scores at the 3 different time-points (24, 48 and 72 h) were combined and averaged. The average irritation scores for the 3 individual rabbits were 1, 1, 0 for corneal opacity; 0.67, 0, 0 for iris irritation; 1.67, 1.67, 1 for conjunctival redness and 1.67, 1, 1 for chemosis. After 7 days, irritation had completely resolved apart from the conjunctival redness in two rabbits, which resolved within 29 days (EU RAR, 2008; REACH).

In another OECD TG 405 eye irritation study, 0.1 g of tonalide was applied to one eye of 3 male NZW rabbits while the other eye served as the control. The irritation scores at the 3 different time-points (24, 48 and 72 h) were combined and averaged. The average irritation scores for the 3 individual rabbits were 0, 0.67, 0 for corneal opacity; 0, 0, 0 for iris irritation; 1, 1.33, 1.33 for conjunctival redness and 1, 1, 1 for chemosis. After 7 days, irritation had completely resolved (EU RAR, 2008; REACH).

Observation in humans

During the induction phase of a human repeat insult patch test (HRIPT), 0.3 mL of 10 % tonalide in ethanol/diethyl phthalate (DEP) (75/25 v/v) was applied to the backs of 111 subjects for 24 h three times per week for 3 weeks. No treatment-related reactions were observed 24 or 72 h after patch removal (EU RAR, 2008).

Tonalide was also negative in two photoirritancy studies in a total of 16 human volunteers at concentrations up to 10 % (EU RAR, 2008).

Sensitisation

Skin Sensitisation

Based on the available data, the tonalide has some sensitisation potential (including photosensitisation) in animals. Both sensitisation and photosensitisation studies in humans were negative (see ***Observation in humans*** section) and there were no structural alerts for skin sensitisation using the OECD Toolbox. The effects in animals are not sufficient to warrant hazard classification.

In a skin sensitisation study in 6 guinea pigs (strain and sex not reported), 0.1 mL of tonalide at 3, 10 or 30 % in ethanol/acetone (50/50 v/v) was applied to 8 cm^2 clipped flank of skin daily for 3 weeks. Due to the cumulative irritation at the site, the application site was changed after 2 weeks. A challenge dose of 0.025 mL of tonalide at 1 % (non-irritating dose) was applied to a naïve site directly after the induction and then again after 2 weeks. No reactions were observed after application of the challenge doses (EU RAR, 2008; REACH).

In another study with limited information available, 8 guinea pigs received intradermal injections of 0.1 mL of 5 % emulsion of tonalide in Freund's complete adjuvant on days 0, 2, 4, 7 and 9. Challenge tests were performed after 3 and 5 weeks by applying 0.025 mL of 5 % tonalide (on a 2 cm^2 skin area (preparation not reported). Dermal reactions were reported in 6/8 guinea pigs at 3 weeks and in 8/8 guinea pigs at 5 weeks; however, it was unclear whether the reactions reported were from test or control animals. No reaction scores were reported. Tonalide was considered a weak intradermal sensitiser (EU RAR, 2008).

The (Q)SAR modelling for skin sensitisation using the OECD QSAR Toolbox (version 4.2) indicated that there were no alerts for skin sensitisation for either the tonalide or its metabolites (skin metabolism and autoxidation).

Photo sensitisation studies

In five (2 GLP; 3 non-GLP) photosensitisation studies in guinea pigs, positive reactions were noted at induction concentrations of 1–10 % and challenge concentrations of 0.3–1 % of tonalide in ethanol. In two other GLP studies in guinea pigs, no reactions were noted at induction concentrations and challenge concentrations of 2 % of tonalide in ethanol. Positive reactions to photo degradation products were also noted in one study. The reactions were less pronounced when a UV filter was applied to the application area prior to irradiation (EU RAR, 2008).

Observation in humans

No sensitisation reactions were seen in three separate human repeated insult patch test (HRIPT) studies at concentrations between 2–10 % in a total of 188 human subjects or in a human patch test study using 1 or 5 % solutions of tonalide in 313 dermatological patients (EU RAR, 2008).

No evidence of photosensitisation was observed in three different photo-HRIPT studies at concentrations between 1–10 % in a total of 54 human subjects (EU RAR, 2008).

Repeated Dose Toxicity

Oral

Tonalide is not expected to cause serious damage to health from repeated oral exposure, based on the reversibility and low severity of the reported effects.

In an oral study conducted according to OECD TG 408, SD rats (15/sex/dose) received daily doses of tonalide in the diet at nominal doses of 1.5, 5, 15 or 50 mg/kg bw/day for 13 weeks. After the treatment period, 3 females and 3 males from the control and high dose groups (recovery group) were maintained without treatment for 4 weeks. No mortalities or clinical signs of toxicity attributable to tonalide were observed during the study. Mean body weight gain was lower in both sexes receiving the highest dose (50 mg/kg bw/day). Males in the high dose group had a significantly reduced bodyweight at week 13; however, this improved during the treatment free period in the recovery group. Food intake was not affected by tonalide.

Relative liver weights were increased in males (32 % increase) and females (13 %) of the highest dose group. At this dose, slight changes in liver enzymes and other liver related blood chemistry changes (protein, glucose, cholesterol and triglcyerides) were also noted. Effects were mainly seen in the high dose groups and were more pronounced in males. These findings were not associated with any histopathological changes in the liver. Mild haematological effects were observed in both sexes at the highest dose and in some animals receiving 15 mg/kg bw/day. Observations in the recovery group indicated that the liver and haematological effects were reversible. Several organs including liver, mesenteric lymph nodes and lacrimal glands were stained green at the higher doses and occasionally at lower doses. These effects were not associated with any histopathological changes and were mostly reversible in the recovery group. A no observed adverse effect level (NOAEL) of 5 mg/kg bw/day was reported based on dose-dependent changes in haematological and biochemistry parameters at 15 and 50 mg/kg bw/day (EU RAR, 2008; REACH).

In a 4 week oral gavage study conducted according to OECD TG 407, Wistar rats (5/sex/dose) were treated with tonalide in corn oil at 0, 1, 3 or 10 mg/kg bw/day. No treatment-related mortality, clinical signs of toxicity, changes in bodyweight or food consumption were observed during the study. No adverse haematological, biochemistry parameters, necropsy and histopathology findings were observed. The NOAEL for the study was 10 mg/kg bw/day (EU RAR, 2008; REACH).

In a 2-week dose-range finding and palatability study, SD rats (5/sex/dose) received the tonalide in the diet. Based on food intake, doses were calculated as 0, 33, 88 and 169 mg/kg bw/day for males and 0, 32, 91 and 150 mg/kg bw/day for females. All animals in the high dose group were sacrificed prematurely on day 5 due to marked reductions in food consumption and bodyweight. Slight reductions in food intake and bodyweights were observed at the mid dose. Increases in relative liver and kidney weights were observed in males and females at the mid and high doses as well as females at the low dose. This was associated with hepatocyte fine vacuolisation in mid dose males and at high doses for both sexes (EU RAR, 2008).

Dermal

Based on the available information, tonalide is not expected to cause serious damage to health from repeated dermal exposure.

In a 13-week non-guideline study, female SD rats (15/dose) received topical applications of tonalide at 1, 10 or 100 mg/kg bw/day as a 1 % (w/v) solution in ethanol. Bodyweights were significantly reduced and liver weights (relative and absolute) were increased at the highest dose (100 mg/kg bw/day). Moderate degrees of hepatocytomegaly, increases in serum alkaline phosphate and decreases in haematological parameters including reduced red blood cell count were also observed at this dose (EU RAR, 2008).

In 26 week non-guideline study, female rats (20/dose) received topical applications of tonalide at 9, 18, or 36 mg/kg bw/day as a 1 % (w/v) solution in ethanol. Similar effects to those seen in the 13 week study were observed. These included increased liver weights (absolute or relative not stated) and reductions in haematological parameters (EU RAR, 2008).

In a 14-week subchronic dermal study, 15 female Wistar rats received topical applications of 100 mg/kg bw/day of tonalide at 10 % in ethanol. The dose was reduced to 10 mg/kg bw/day at day 8 due to severe irritation and complete inhibition of body weight gain. The reduced body weight gain was attributed to the severe irritation. After the treatment period, 5 rats remained without treatment for a 6 week recovery period. The skin gradually recovered and weight gain was normal one week after lowering the dose. Dark colouration of the kidneys were noted in 3/10 rats; however, this was reversible (EU RAR, 2008).

No measures were taken to prevent oral ingestion of tonalide in the dermal studies described above. Therefore, it is not possible to determine the actual exposure to or NOAEL for tonalide. No further information is available for these studies.

Inhalation

No data are available.

Genotoxicity

Based on the available data tonalide is not expected to be genotoxic (EU RAR, 2008; REACH).

In vitro

Tonalide was negative in:

- point mutation studies in *Salmonella typhimurium* strains TA97, TA98 TA100, TA102, TA1535, 1537 and the *Escherichia coli* WP2 uvrA strain at concentrations up to 5000 µg/plate, with and without metabolic activation;
- a chromosome aberration assay in Chinese hamster ovary (CHO) cells at concentrations up to 23.4 µg/mL for 4, 20 and 44 h without metabolic activation, and at concentrations up to 25 µg/mL for 20 h and up to 15.6 µg/mL for 44 h with metabolic activation;
- two sister chromatid exchange (SCE) assays in human lymphocytes at concentrations up to 40 µg/mL or 97 µM for 24 h, with or without metabolic activation;
- two micronucleus tests in human peripheral lymphocytes and human hepatoma cells at concentrations up to 194 µM for 48 h, with or without metabolic activation; and
- an in vitro unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes at concentrations up to 15 µg/mL.

In vivo

Tonalide was negative in a OECD TG 474 micronucleus test. No significant increases in micronucleated polychromatic erythrocytes of Swiss albino (ICR) mice (5/sex/dose) were observed at 24, 48 h and 72 h after intraperitoneal administration of tonalide at 400, 800, or 1600 mg/kg bw.

Carcinogenicity

Data from long term oral studies are not available. Tonalide is not considered genotoxic (see ***Genotoxicity*** section) and has no liver tumour initiating and promoting activity in rats.

In a liver tumour initiating and promoting study, Wistar rats (54/sex/dose) were exposed to 300 µg/kg bw/day alone or to a single dose of diethylnitrosamine (100 mg/kg bw) followed by tonalide at 1, 10, 100 or 300 µg/kg bw/day via intraperitoneal injection for 90 days. There was no evidence of tumour initiating or promoting activity effect on the livers of any of the animals (EU RAR, 2008; REACH).

Reproductive and Developmental Toxicity

Tonalide does not show specific reproductive or developmental toxicity.

No effect on reproductive organs were reported in a 13-week oral toxicity study (see ***Repeated dose toxicity*** section) at doses up to 50 mg/kg bw/day. Developmental effects were only observed secondary to maternal toxicity.

In a developmental toxicity study conducted similarly to OECD TG 414, pregnant SD rats (25/dose) were orally administered tonalide by gavage at 0, 5, 15 or 50 mg/kg bw/day in corn oil on days 7–17 of pregnancy. Initial weight loss in the high dose (50 mg/kg bw/day) group was followed by significant reduction in maternal body weight gains (23.5 %). Food consumption was reduced by 23 % in animals receiving the high dose and by 8 % in the mid dose (15 mg/kg bw/day) animals. No other effects were observed in the mid dose group. The NOAEL for maternal toxicity was 15 mg/kg bw/day. The average foetal weight was slightly reduced in all dose groups; however, the effects were not dose-dependent and were within the historical range. There were no effects observed on numbers of implantations, foetuses, or resorptions. All gross external, soft tissue or skeletal malformations in the foetuses were reported as incidental and not attributed to tonalide. Therefore, the NOAEL for developmental toxicity is 50 mg/kg bw day (EU RAR, 2008; REACH).

In a preliminary developmental toxicity study, pregnant SD rats (8/dose) were orally administered tonalide by gavage at 10, 25, 50 or 100 mg/kg bw/day in corn oil on days 7–17 of pregnancy. Weight loss was recorded in the highest dose group and body weight gain was reduced at doses of 25 and 50 mg/kg bw/day. Food intake was reduced at the two highest doses. Litter parameters were unaffected and no external alterations were reported at doses 50 mg/kg bw/day and lower. Three foetuses in the high dose group had whole body oedema (EU RAR, 2008).

In a neonate nursing study, pregnant SD rats (28/dose) were orally administered tonalide by gavage at 0, 2, 6 or 20 mg/kg bw/day from day 14 of pregnancy through to weaning on day 21 after birth. There were no treatment related effects in any of the treated parent females (F0) during pregnancy or lactation. The females were allowed to produce a litter and from these litters 24 males and females (F1 generation) were retained to maturity and assessed for behavioural changes and reproductive capacity. The F1 generation were only exposed to tonalide in utero and through mother's milk. No adverse effects were noted on the development of the F1 generation during the late prenatal phase or on postnatal growth. After approximately 84 days of observations, the F1 generation were mated. Reproductive capacity and litter size in the F1 generation were normal. The F2 generation was observed until 21 days after birth. No changes in post weaning behavioural tests or mating performance were observed. No abnormalities were seen in the F2 pups. The amount of tonalide in mothers' milk was not measured in the study. However, the level of tonalide in the milk can be estimated to be ~10 µg/mL after daily dosing of 20 mg/kg bw/day, based on results from a pharmacokinetic study in lactating rats (see ***Toxicokinetics*** section) (EU RAR, 2008; REACH).

Other Health Effects

Endocrine Disruption

In vitro and zebrafish studies suggest that tonalide has weak endocrine activity by binding to steroid hormone receptors. However, no effects were seen in an in vivo uterotrophic assay in mice. At this stage there is no evidence of these weak endocrine activities causing adverse effects in mammals or humans.

In vitro

Tonalide had a very weak agonism towards the human oestrogen receptor (hER) alpha and weak antagonism towards hER beta in an in vitro assay using human embryonic kidney 293 (HEK293) cells stably transfected with the ERs. The effects were only seen at high concentrations (10 µM) of tonalide. Similar agonistic effects have been demonstrated in other studies but only at concentrations 10000 times higher than for the natural ligand 17-beta-oestradiol (EU RAR, 2008; Witorsch et al., 2010).

Tonalide has weak anti-androgenic potential but is at least 20 times less potent than the androgen antagonist vinclozolin and >10000 times less potent than the endogenous agonist dihydrotestosterone (EU RAR, 2008; Witorsch et al., 2010).

In another study tonalide demonstrated weak antagonism towards the progesterone receptor (PR); however, it was 4000 times less potent than the anti-progestogenic drug mifepristone (Schreurs et al., 2005).

In vivo

In a 2-week uterotrophic assay, female Balb/c mice (6/dose) received 10 or 50 mg/kg bw/day of tonalide in the diet. Tonalide had no significant effects on uterine weights, indicating that tonalide does not have oestrogenic activity in mice (EU RAR, 2008; Seinen et al., 1999).

In a study in transgenic zebrafish expressing zebrafish ER beta and gamma, tonalide did not show any oestrogenic effects at concentrations of 0.01, 0.1 and 1 μM. Dose-dependent antagonistic effects were observed at concentrations of 0.1 and 1 μM (EU RAR, 2008; Schreurs et al., 2005).

Risk Characterisation

Critical Health Effects

The critical health effect for risk characterisation is acute toxicity from oral exposure.

Public Risk Characterisation

Considering the range of domestic, cosmetic and personal care products that could contain the chemicals, the main route of public exposure is expected to be through the skin, inhaled from products applied as aerosols, and potential oral exposure from lip and oral hygiene products. Infants could also be exposed to the chemicals via breast milk.

Breast-fed infants could be exposed through mother's milk; however, the concentrations are expected to be very low. The difference in the estimated uptake concentrations (see ***Toxicokinetics*** section) between a rat study (where no adverse effects were found) compared to estimated infant exposure is approximately 1000 (EU RAR, 2008). This margin of safety indicates that tonalide, when transferred via breast milk, does not pose a health risk to infants.

Concentrations in cosmetic and domestic products are expected to be low (EU RAR, 2008; see ***International Restrictions*** section). The available data do not indicate any hazards associated with exposure to tonalide at these low concentrations.

Tonalide has very weak endocrine activity in vitro; however, no effects were seen in an in vivo study in mice.

Should additional information associated with the weak endocrine activity become available, further assessment of the chemicals may be required.

Occupational Risk Characterisation

During product formulation, oral exposure might occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to the chemicals at lower concentrations could also occur while using formulated products containing the chemicals. The level and route of exposure will vary depending on the method of application and work practices employed.

Given the critical systemic acute health effects, the chemicals could pose an unreasonable risk to workers unless adequate control measures to minimise oral exposure are implemented. The chemicals should be appropriately classified and labelled to ensure that a person conducting a business or undertaking (PCBU) at a workplace (such as an employer) has adequate information to determine the appropriate controls.

The data available support an amendment to the hazard classification in the HCIS (Safe Work Australia) (see ***Recommendation*** section).

NICNAS Recommendation

Assessment of the chemicals are considered to be sufficient, provided that the recommended amendment to the classification is adopted, and labelling and all other requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory.

Tonalide has been shown to have weak endocrine activity. However, the available data do not demonstrate potential to cause adverse effects via this endocrine activity. NICNAS will continue to monitor the availability of high quality data emerging on tonalide to determine if further assessment may be required.

Regulatory Control

Work Health and Safety

The chemicals are recommended for classification and labelling aligned with the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) as below. This does not consider classification of physical hazards and environmental hazards.

From 1 January 2017, under the model Work Health and Safety Regulations, chemicals are no longer to be classified under the Approved Criteria for Classifying Hazardous Substances system.

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Acute Toxicity	Not Applicable	Harmful if swallowed - Cat. 4 (H302)

[a] Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)].

[b] Globally Harmonized System of Classification and Labelling of Chemicals (GHS) United Nations, 2009. Third Edition.

[*] Existing Hazard Classification. No change recommended to this classification

Advice for industry

Control measures

Control measures to minimise the risk from oral exposure to the chemicals should be implemented in accordance with the hierarchy of controls. Approaches to minimise risk include substitution, isolation and engineering controls. Measures required to eliminate, or minimise risk arising from storing, handling and using a hazardous chemical depend on the physical form and the manner in which the chemical is used. Examples of control measures that could minimise the risk include, but are not limited to:

- minimising manual processes and work tasks through automating processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemicals.

Guidance on managing risks from hazardous chemicals are provided in the *Managing risks of hazardous chemicals in the workplace—Code of practice* available on the Safe Work Australia website.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

Obligations under workplace health and safety legislation

Information in this report should be taken into account to help meet obligations under workplace health and safety legislation as adopted by the relevant state or territory. This includes, but is not limited to:

- ensuring that hazardous chemicals are correctly classified and labelled;

- ensuring that (material) safety data sheets ((M)SDS) containing accurate information about the hazards (relating to both health hazards and physicochemical (physical) hazards) of the chemicals are prepared; and
- managing risks arising from storing, handling and using a hazardous chemical.

Your work health and safety regulator should be contacted for information on the work health and safety laws in your jurisdiction.

Information on how to prepare an (M)SDS and how to label containers of hazardous chemicals are provided in relevant codes of practice such as the *Preparation of safety data sheets for hazardous chemicals—Code of practice* and *Labelling of workplace hazardous chemicals—Code of practice*, respectively. These codes of practice are available from the Safe Work Australia website.

A review of the physical hazards of the chemicals has not been undertaken as part of this assessment.

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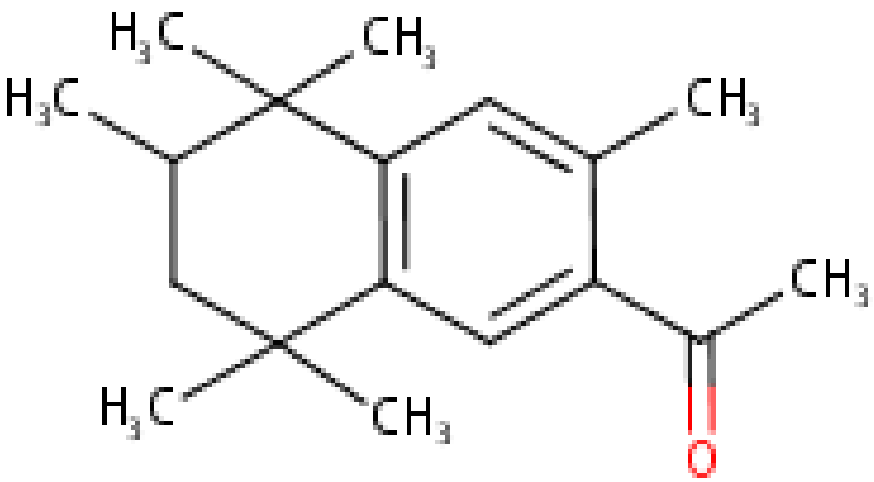
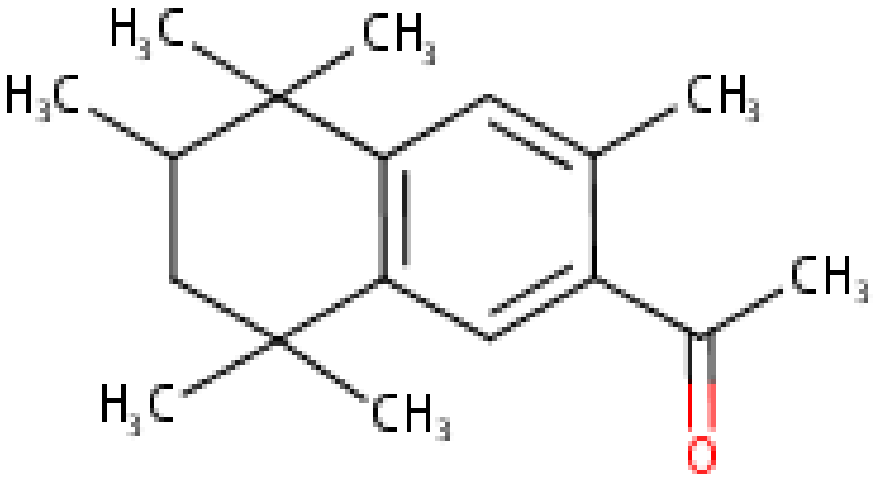
Last Update 08 March 2019

Chemical Identities

Chemical Name in the Inventory and Synonyms	**Ethanone, 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)-** 7-Acetyl-1,1,3,4,4,6-hexamethyltetrahydronaphthalene 7-Acetyl-1,1,3,4,4,6-hexamethyltetralin AHTN Tonalide
CAS Number	1506-02-1
Structural Formula	
Molecular Formula	C18H26O
Molecular Weight	258.40

Chemical Name in the Inventory and Synonyms	**Ethanone, 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)-** 7-Acetyl-1,1,3,4,4,6-hexamethyltetrahydronaphthalene 7-Acetyl-1,1,3,4,4,6-hexamethyltetralin AHTN Tonalide

CAS Number	21145-77-7
Structural Formula	
Molecular Formula	C18H26O
Molecular Weight	258.40

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