2-Butanone, 1-(4-chlorophenoxy)-1-(1H-imidazol-1-yl)-3,3-dimethyl-: Human health tier II assessment

05 February 2016

CAS Number: 38083-17-9

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.

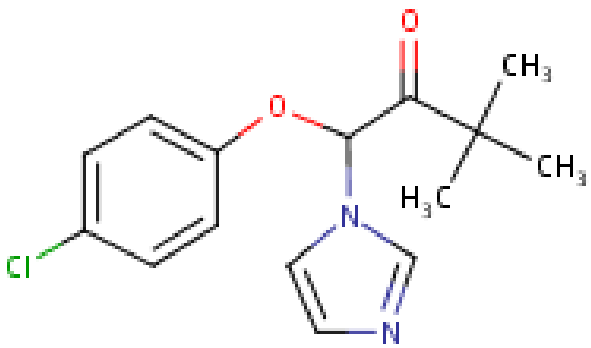
This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

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

Disclaimer

Acronyms & Abbreviations

Chemical Identity

Synonyms	1-[(4-chlorophenoxy)(tert-butylcarbonyl)methyl 1-(4-chlorophenoxy)-1-(1H-imidazolyl)-3,3-dimethyl-2-butanone climbazole crinipan AD baypival
Structural Formula	
Molecular Formula	C15H17ClN2O2
Molecular Weight (g/mol)	292.76
Appearance and Odour (where available)	Solid, powder, crystals, white to pale brown, crystalline powder.

SMILES	C(C)(C)(C)C(=O)C(N1C=CN=C1)Oc1ccc(Cl)cc1

Import, Manufacture and Use

Australian

No specific Australian industrial use, import, or manufacturing information has been identified.

International

The following international uses have been identified through:

- the European Union (EU) Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) dossiers;
- Galleria Chemica;
- the European Commission Cosmetic Ingredients and Substances (CosIng) database;
- the United States (US) Personal Care Products Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary; and
- various international assessments (SCCP, 2005; SCCP, 2008).

The chemical has reported cosmetic uses as a preservative and an antimicrobial agent in:

- shampoos;
- cleansing products (cold creams, lotions, liquids and pads);
- moisturising preparations;
- skin care preparations;
- tonics;
- dressings; and
- other hair growing aids.

The chemical has reported non-industrial uses including in pesticides and in therapeutic products (antifungal).

Restrictions

Australian

This chemical is listed in the *Poisons Standard—the Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP) in Schedules 5 and Schedule 6 (SUSMP, 2015).

Schedule 6:

CLIMBAZOLE **except**:

a) when included in Schedule 5; or

b) in preparations containing 2 per cent or less of climbazole.

Schedule 6 chemicals are described as 'Substances with a moderate potential for causing harm, the extent of which can be reduced through the use of distinctive packaging with strong warnings and safety directions on the label'. Schedule 6 chemicals are labelled with 'Poison' (SUSMP, 2015).

Schedule 5:

'CLIMBAZOLE in preparations containing 40 per cent or less of climbazole except in preparations containing 2 per cent or less of climbazole.

Schedule 5 chemicals are described as 'Substances with a low potential for causing harm, the extent of which can be reduced through the use of appropriate packaging with simple warnings and safety directions on the label.' Schedule 5 chemicals are labelled with 'Caution' (SUSMP, 2015).

International

The chemical is listed on the following (Galleria Chemica):

- Costa Rica Prohibited and Restricted Pesticides;
- Sweden Restricted Substances Database; and
- United Arab Emirates Restricted Chemicals;

In the Association of South East Asian Nations (ASEAN) (including the Philippines), Chile and New Zealand, the maximum allowed concentration of the chemical as preservative in cosmetic products is 0.5 %.

In the European Union (EU), the chemical is listed in the Cosmetics Directives as a preservative in Annex VI, with a maximum authorised concentration of 0.5 % in leave-on hair and face cosmetics and 2 % for rinse-off hair cosmetics.

Existing Work Health and Safety Controls

Hazard Classification

The chemical is not listed on the Hazardous Substances Information System (HSIS) (Safe Work Australia).

Exposure Standards

Australian

No specific exposure standards are available.

International

No specific exposure standards are available.

Health Hazard Information

The chemical, referred throughout this assessment as climbazole, could contain impurities including: 4-chlorophenol (CAS No 106-48-9), xylenes; hexane; 2-ethylhexanol; benzene; toluene; cyclohexane; chlorine; and octane (SCCP, 2008; USP, 2011). The potential of some of these impurities to influence the toxicity of climbazole cannot be ruled out.

Toxicokinetics

The toxicokinetics of the chemical have been investigated in laboratory animals.

In an oral study in CD-1 mice, the chemical was found to be rapidly absorbed and excreted following oral exposure. In this guideline-compliant study, male CD-1 mice (n=21) were given a single dose of 150 mg/kg bw radiolabelled climbazole. Analyses of the plasma for radioactivity were performed in three animals at 15 mins; 30 mins; and 1, 2, 4, 8, and 24 hours. The earliest radioactivity detection, with mean concentration of 20 µg equivalent/g, was 15 minutes after dose administration. The highest mean concentration of radioactivity in the plasma observed eight hours after dose administration was 20.4 µg equivalent/g. This level was increased to 46.6 µg equivalent/g after 24 hours (SCCP, 2008; REACH).

The results from a subchronic oral study in beagle dogs indicated that climbazole is rapidly metabolised to BAY g 5919 (REACH). In this study, dogs (n=3) were given gelatin capsules containing 0, 5, 10, or 20 mg/kg bw climbazole daily for 13 weeks. The four-hour plasma level of BAY g 5919 metabolite was found to exceed by a factor of two to five that of the parent chemical. The total concentration of BAY g 5919 metabolite and the parent chemical was less than 10 ng/mL 24 hours after dosing (REACH).

In an earlier study, predating the establishment of good laboratory practice (GLP), the chemical was reported to be rapidly metabolised and excreted by Wistar rats following a single oral exposure (gavage) of 50 mg/kg dose (REACH). The peak plasma concentration was observed 30 minutes after dosing. The estimated plasma half-life was three to four hours after dosing. In another study, the chemical was completely excreted after 16 hours. No details were provided on the route of exposure (REACH).

The dermal absorption of climbazole has been investigated in vitro in pigs and humans, conducted in accordance with the Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 428. The following climbazole formulations in cosmetic products were used: 2 % in shampoos for the human skin study; and 0.5 % in the hair serum or skin serum formulations for the pig skin study (SCCP, 2008). For the human skin study, radiolabelled climbazole was applied to 12 dermatomed 400 µm-thick human skin samples. In the pig skin study, radiolabelled climbazole was applied to 12 dermatomed 400 µm-thick pig skin samples. Using the static Franz diffusion cells, climbazole was applied to human and pig skin (10 mg/cm^2 area). After 30 minutes, the chemical was washed off and receptor fluid concentrations were evaluated at 0, 0.5, 1, 2, 4, 6, and 24 hours. Under these experimental conditions, the chemical was systematically bioavailable at 0.15 % or 0.297µg/cm^2 in the human skin study. In pigs, climbazole was bioavailable at 2.23 % or 1.10 µg/cm^2 from the hair serum and 3.46 % or 1.25 µg/cm^2 from the skin serum (SCCP, 2008).

The dermal absorption of the chemical was also examined in humans in vivo in four non-guideline studies (SCCP, 2005). In these studies, 0.5 % of climbazole in isopropanol/water, 2.0 % of climbazole in shampoo and 1.0 % of climbazole in hair lotion were applied to the skin of male and female volunteers. The concentrations were measured at 1, 2, 4, 8, and 24 hours following applications. Based on the findings, the chemical or its metabolite (approximately 3-34 ng/mL) were detected in blood plasma and urine (SCCP, 2005).

The chemical was reported to be a potent inducer and/or inhibitor of hepatic microsomal P450 metabolising enzymes (Kobayashi et al., 2002).

Acute Toxicity

Oral

The acute oral toxicity of climbazole has been investigated in earlier studies (predating GLP; conducted similarly to OECD TG 401) in rats, mice, rabbits and dogs. Based on the results, the chemical has moderate acute toxicity following oral exposure. The

median lethal dose (LD50) is 400 mg/kg bw in rats. The chemical is recommended for classification (see **Recommendation** section).

Climbazole, suspended in 5 % Cremophor and tap water, was administered to Wistar rats (n=10) and male CF1/W 68 mice (n=10) by stomach tube at the following doses: 0, 160, 200, 250, 320, 400, 500, 630, 800 or 1000 mg/kg bw in rats; and 0, 320, 400, 500, 630, 800 or 1000 mg/kg bw in mice. The animals were observed for treatment-related effects for 14 days. Probit analysis (a statistical method to measure dose-response relationships) was used to calculate the LD50 of climbazole in this study (REACH). In rats, mortality occurred within 24 hours at 200-1000 mg/kg bw and few more animals died within seven days. Sublethal effects observed an hour after exposure include disturbances in body coordination and, except for the 160 mg/kg bw group, seizure of the entire body (tonic-clonic convulsions). Dose-dependent changes, including reduced movement, were noted two hours after exposure. During this time, the animals were transiently lying on their abdomen. These effects were reversible within 48 hours after exposure. Under these conditions, the chemical was considered moderately toxic with a reported LD50 of 400 mg/kg bw (REACH). In mice, death occurred mainly one hour after treatment: 2/10 at 400 mg/kg bw; 3/10 at 500 mg/kg bw; 2/10 at 630 mg/kg bw; 6/10 at 800 mg/kg bw; and 10/10 at 1000 mg/kg bw. The animals that survived displayed reduced movement and showed tonic-clonic convulsions within 10 minutes of exposure. The intensities of the effects were dose-dependent. The LD50 derived from this study was 664 mg/kg bw (SCCP, 2005; REACH).

Female Chinchilla rabbits (n=2 per dose) were administered 10 mL of solution containing 0, 125, 250 and 500 mg/kg bw climbazole suspended in tap water and 5 % Cremophor. Effects were observed for 14 days. Death occurred in one rabbit in the 250 mg/kg bw group 24 hours after exposure, with no observed symptoms prior to death. The other animal in the group appeared sedated and was observed lying on the belly 24 hours after exposure. This effect was no longer seen after 48 hours. Animals treated at 500 mg/kg bw died within 24 hours after exposure, with some degree of sedation noted prior to death. The LD50 derived from this study was 250 mg/kg bw (SCCP, 2005; REACH).

Male and female beagle dogs (n=2 per dose) were administered gelatin capsules containing 50, 100, 250 or 500 mg/kg bw of the chemical. In the 100 mg/kg bw group, the animals displayed ataxia at 24 hours but this effect was no longer noted 48 hours after exposure. In the 250 mg/kg bw group, one animal was in a lateral recumbent position (lying on the side) with slight convulsions and died within 30 hours after exposure. In the 500 mg/kg bw, one animal vomited within three hours of treatment and died 24 hours later. The other animals in the 250 and 500 mg/kg bw groups did not show any treatment-related effects. The LD50 value reported in this study was in the range of 250-500 mg/kg bw (SCCP, 2005; REACH).

Dermal

The chemical has low acute toxicity based on results from animal tests following dermal exposure. The median lethal dose (LD50) in rats is >5000 mg/kg bw. No study details were supplied in the publicly available documents (SCCP, 2008).

Inhalation

No data are available.

Corrosion / Irritation

Skin Irritation

The skin irritation potential of climbazole has been investigated in vitro and in vivo in rabbits and also in humans (see **Observations in humans** section). Based on the data from available studies, climbazole is slightly irritating to the skin of rabbits in vivo and non-irritating in vitro in a human skin model.

The chemical was reported to be slightly irritating to the skin of New Zealand White (NZW) rabbits following application of the chemical as a solution at concentrations of 0.5 % in hair lotion or 0.5 % in emulsion with polyethylene glycol 400 (vehicle), for seven hours once daily (washed after each treatment), five days a week for three weeks. However, this study was non-guideline compliant and was conducted prior to the establishment of GLP (REACH).

Skin irritation was also observed following exposure to climbazole in a non-guideline skin sensitisation study in female Bor:DHPW guinea pigs. In this study, 10 % climbazole appeared to cause skin irritation (see **Sensitisation: skin** section).

In an in vitro test using a reconstructed human epidermis model, the EpiDerm™ test, 22.7 mg neat climbazole was applied to human skin tissues for 60 minutes. In this guideline-compliant test (EU Test Method B.46), the effects were measured using the methylthiazolyldiphenyl-tetrazolium (MTT) assay, a colorimetric assay to measure viability of cells. Cells with active or functioning metabolism are capable of reducing MTT into formazan that absorbs light at a wavelength of 570nm (van Merlo et al., 2011). Following exposure to climbazole, the mean absorbance value (indicating viability), as measured by a spectrophotometer, was reported to be well above the value that indicates the threshold for irritation (REACH).

Eye Irritation

The eye irritation potential of climbazole has been tested in vivo in rabbits and in vitro using bovine and avian eyes. Based on the results, the chemical is not considered an eye irritant.

Climbazole was found to be non-irritating in the eyes of white rabbits (n=8; strain not specified) in an early primary irritation study (predating GLP). In this study, 0.1 mL of emulsion containing 0.5 % climbazole (in polyethylene glycol 400) was instilled into the conjuctival sac of one eye of each rabbit for five minutes or 24 hours (SCCP, 2005). The exposed eyes were washed after five minutes or 24 hours by rinsing with water for two minutes. Effects were monitored for seven days. The results showed moderate, transient reddening and slight swelling of the eye in one animal after the five-minute exposure. Slight reddening of the eye was also sporadically observed in other animals, including those exposed for 24 hours. Considering the non-guideline compliant nature of the study (i.e. eyes were washed after five minutes and scoring system not specified), the regulatory value of this study is limited (SCCP, 2005).

The in vitro bovine corneal opacity and permeability (BCOP) test was conducted to assess the eye irritation potential of climbazole. In this OECD TG 437-compliant study, 289 mg neat chemical was applied directly to the cornea of enucleated cow eyes for 10 minutes. Under the conditions of the test, the chemical is not considered an eye irritant (REACH).

In another in vitro study, the chemical did not cause eye irritation when tested in the Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) assay (SCCP, 2008). In this study, 15 fertilised eggs from the Lohmann Selected Leghorn chicken were opened following nine days of incubation. Neat climbazole was applied to six of the eggs at 100 mg/kg for 300 seconds. This exposure period guarantees at least 25 % membrane coverage. The study used 0.9 % sodium chloride (NaCl) and 1 % sodium dodecyl sulfate (SDS) as negative and positive controls, respectively. The eggs were evaluated for haemorrhage, coagulation and blood vessel lysis. In these conditions, no climbazole-induced effects were reported (SCCP, 2008).

In another in vitro assay, the Chicken Enucleated Eye Test (CEET), no irritation was reported after exposing the eyes to climbazole (SCCP, 2008). In this OECD TG 438-compliant test, 0, 0.5, 1.0 and 2.0 % of the chemical suspended in propylene glycol, was applied onto the isolated chicken eyes (from the slaughter house) for 10 seconds. The climbazole-exposed eyes were examined for the following: swelling and opacity of the cornea; retention of fluorescein by damaged epithelial cells; and histopathology. The positive and negative controls used in this study were SDS and polyethylene glycol, respectively. Effects observed in the climbazole-treated eyes were also noted in the control group. These include slight corneal swelling and opacity, slight fluorescein retention and slight epithelial erosions (SCCP, 2008).

Observation in humans

Patch tests were used to investigate the skin irritation potential of climbazole in human volunteers. In these tests, the chemical was not considered a skin irritant at 2 % (SCCP, 2008).

In one patch test, 0.3 mL of 2 % climbazole (in ethanol and water) was applied to the skin of 21 volunteers as semi-occlusive patches for 24 hours. The positive and negative controls used were sodium laurel sulfate and distilled water, respectively. Effects were evaluated at 30 and 60 minutes or 24 hours after removing the patches. Under these conditions, 2 % climbazole did not produce signs of skin irritation (SCCP, 2008).

In another patch test, 2 % of the chemical (in an antidandruff shampoo) was applied to the skin of 49 volunteers, under semi-occlusive conditions for 24 hours. This study used SDS and demineralised water as positive and negative controls, respectively. The skin sites were graded for irritation at 24 or 25 hours (first evaluation) and 48 hours (second evaluation) after application of

the chemical. At the first evaluation, 1/49 volunteers showed slight irritation. No skin reaction was reported at the second evaluation (REACH).

Sensitisation

Skin Sensitisation

The chemical was not found to induce dermal sensitisation when tested in mouse local lymph node assay (LLNA), guinea pig maximation test (GMPT), and Buehler test.

In the LLNA (OECD TG 429-compliant), the dorsal surface of each ear of CBA/J mice were exposed to 1, 5, 10, or 20 % climbazole once daily for three consecutive days. The positive control used in this study was 35 % a-hexylcinnamaldehyde. Under the conditions of the test, the chemical did not cause dermal sensitisation up to 20 %. The stimulation indices (SI) reported were 0.91; 0.76; 1.19; and 1.08 respectively. The SI value for the positive control was 7.77 (SCCP, 2008; REACH).

A Buehler test (OECD TG 406 non-compliant), using three applications of climbazole, was undertaken in 15 female Hartley guinea pigs. Induction was performed using 10 % of climbazole in carboxymethyl cellulose (CMC), applied epicutaneously under occlusive condition for 24 hours. The positive control used in this study was 1-tetradecene-1,3-sultone suspended in diethyl either solution. The challenge used 0.3, 1 and 3 % climbazole in CMC, on the shaved back skin of animals, for 24 hours. Effects were evaluated three hours after the challenge exposure. Under conditions of the test, the chemical did not produce significant skin sensitisation in female guinea pigs (REACH).

A GMPT (OECD TG 406 non-compliant) was conducted in female Bor:DHPW guinea pigs. The first intradermal induction used 10 % of climbazole suspended in 1,2 propanediol and Freund's Complete Adjuvant. The second topical induction also used 10 % of climbazole in 1,2 propanediol applied to the skin of animals under occlusive conditions for 48 hours. The animals were challenged at days 21 and 35 with an epicutaneous application of either 10 and 1 % (challenge phase one) or 1 and 0.1 % (challenge phase two) of the chemical. The results showed skin reactions in animals in both the treated and nontreated control groups. Hence, this observation was considered a result of primary irritation. Overall, the study demonstrates that climbazole is not a skin sensitiser up to 10 % in guinea pigs (REACH).

Repeated Dose Toxicity

Oral

Based on the absence of treatment-related effects reported in various repeated dose toxicity studies, repeated oral exposure to the chemical is not considered to cause serious damage to health.

In a pre-GLP 28-day repeat dose oral study in male Wistar rats (n=10/group), a lowest observed adverse effect level (LOAEL) of 50 mg/kg bw/day was reported (SCCP, 2005; REACH). The chemical was given to rats by oral gavage at concentrations of 0, 50, or 100 mg/kg bw/day for 28 days. The animals were monitored for treatment-related effects including changes in clinical chemistry and haematogical parameters, urinalysis and histopathology. At necropsy and histopathology, the following degenerative changes in the liver were observed in all treated animals: pale discolouration with clear lobe delineation; and some degree of intermediate-peripheral or diffuse fatty degeneration (SCCP, 2005; REACH). At 50 mg/kg bw/day, the animals showed significant increase in the enzymatic activity of alanine aminotransferase. Significant elevation of the absolute and relative weights of the thyroids were noted at 100 mg/kg bw/day.

In a 90-day repeat dose study predating GLP, male and female Wistar rats (n=15/sex/dose) were treated daily with 0, 5, 15 or 45 mg/kg bw/day climbazole suspended in 0.5 % aqueous Tylose by oral gavage (SCCP, 2005; REACH). The findings indicated reduction of alkaline phosphatase activity in females in all dosed groups. At 45 mg/kg bw/day, reduced creatinine levels in both sexes and reduced number of erythrocytes in males were reported. The reduction in creatinine levels were within historical controls and the decreased erythrocyte counts were not considered to be related to treatment. Changes in absolute liver weight were significant at doses of 15 and 45 mg/kg bw/day. These changes were associated with increased enzymatic activities of N-demethylase and cytochrome P450. Thus, the observed changes were considered to be an adaptive response rather than

chemical-induced liver toxicity. No deaths were reported in this study. The no observed adverse effect level (NOAEL) in this study was established at a conservative value of 5 mg/kg bw/day (SCCP, 2005; REACH).

In a repeat dose oral study in beagle dogs (non-guideline, predating GLP), gelatin capsules containing 100 mg/kg bw/day of the chemical were given to the animals daily for the first two weeks of the study and the dose was reduced to 50 mg/kg bw/day for the remaining two weeks. Clinical chemistry, haematology, urinalysis and climbazole levels were recorded before and after the treatment. Two weeks after the last treatment, the animals were sacrificed and effects were evaluated. No treatment-related deaths were reported during the course of the study. Within the first two weeks of treatment, signs of toxicity were noted including anorexia and sedation. When the dose was reduced to 50 mg/kg bw/day, these effects were reversed. Other treatment-related changes reported were elevated level of the liver enzyme alanine aminotransferase, and slight anaemia (SCCP, 2005).

In another study in dogs (non-guideline, predating GLP), gelatin capsules containing 0, 5, 10, or 20 mg/kg bw/day climbazole were given to beagle dogs daily for 13 weeks. Signs of toxicity were monitored daily. Additionally, the treated animals were examined for haematological, clinical-chemical, and neurological changes during 2, 5, and 12 weeks of the study. The animals were tested for ophthalmoscopic changes at 5 and 12 weeks of the study. Recordings of electrocardiograms of all animals were conducted one hour before and after the 20th, 43rd and 92nd treatment. Histopathological analysis was conducted in a number of organs of treated animals. The results indicated no treatment-related changes in any of the parameters tested (SCCP, 2005).

Dermal

No data are available.

Inhalation

Based on the information available, repeated inhalation exposure to the chemical is not considered to cause serious damage to health.

In one study, male and female Wistar rats were exposed to 0, 17.2, 44.3 or 104.7 mg/m^3 climbazole in an inhalation chamber for six hours/day, five days/week for three weeks (REACH). Although this study was prior to the establishment of GLP, it was conducted using protocols equivalent to OECD TG 412. The animals were examined for treatment-related effects on body weight, histopathology and clinical chemistry parameters. There were no deaths in any of the treated animals. In males rats treated with 104.7 mg/m^3, the following observations were reported: significant reduction in body weight; significant reduction in absolute heart weights; and increase in the relative weights of the testes, lung, liver and the thyroid. The females in this dose group also showed increases in the absolute and relative liver and adrenal weights. Males from 17.2 and 44.3 mg/m^3 groups had reduced number of leukocytes. At 44.3 mg/m^3, a significant increase in relative thyroid weights was reported in females. However, this was not considered toxicologically relevant due to the absence of absolute weight changes and of similar effects in males (REACH). Changes in glucose, creatinine and alkaline phosphatase levels were noted in the climbazole-treated rats but were not considered treatment-related because they were within the normal physiological range. The no observed adverse effect concentration (NOAEC) derived from this study was 44.3 mg/m^3 (REACH).

In another pre-GLP three-week inhalation study in Wistar rats, a lowest observed adverse effect concentration (LOAEC) of 69.1 mg/m^3 was reported (REACH). In this study, rats were exposed to 0, 69.1 (low dose), 144.4 (mid dose), and 377.1 mg/m^3 (high dose) of the chemical in dynamic inhalation chambers. No deaths were reported but changes in clinical chemistry parameters (liver enzymes and glucose levels) were reported in animals from the mid and high dose groups. However, these effects were not considered toxicologically relevant because the levels were within the normal physiological range. The males in these dose groups showed significantly lower relative spleen weights and the females had higher liver weights. Compared with controls, the relative weights of adrenals and ovaries in females were higher in low, mid and high dose groups. Additionally, the females from the low dose group had higher relative weights of thyroids, heart, lung, and kidneys (REACH).

Genotoxicity

Based on the results from the available in vitro and in vivo genotoxicity studies, the chemical is not considered genotoxic. Some in vitro genotoxicity tests indicated positive results, but all in vivo tests were negative.

The chemical gave negative results in the following guideline compliant in vitro tests:

- bacterial gene mutation test using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 with or without metabolic activation at concentrations up to 5000 µg/plate (OECD TG 471);
- bacterial gene mutation test using *Escherichia coli* strain WP2 uvrA (pKM101) with or without metabolic activation at concentrations up to 5000 µg/plate (OECD TG 471); and
- mammalian cell micronucleus test in human peripheral blood lymphocytes with or without metabolic activation for up to 200 µg/mL (OECD TG 487) (SCCP, 2008).

The negative results descibed above were consistent with the findings from three early non-guideline compliant pre-GLP in vitro tests. In these studies, Ames tests were performed to determine the mutagenicity of the chemical and its metabolite BAY g 5919 in *S. typhimurium* strains TA98, TA100, TA1537 (highest concentration tested was 2000 µg/plate) and *E. coli* strain WP2 uvrA (highest concentration tested was 5000 µg/plate) with or without metabolic activation (SCCP, 2005). Although one study reported a slight toxic effect at 2000 µg/plate of climbazole (with metabolic activation), the overall result indicated that the chemical was not mutagenic under these test conditions (SCCP, 2005). In another non guideline compliant in vitro test (mammalian chromosome aberration test) in human lymphocyte culture, 0, 25, 50, and 100 µg/mL climbazole was negative for chromosomal aberration (SCCP, 2005).

In a most recent OECD TG 476-compliant in vitro test, the chemical tested positive in the L5178Y mouse lymphoma cell line $tk^{+/-}$ gene mutation test (SCCP, 2008). This study evaluated concentrations from 0.5 to 3000 µg/mL, with exposure periods of four and 24 hours in the presence or absence of metabolic activation. The positive controls used in this study were methyl methanesulfonate and 7,12-dimethyl-benz(a)anthracene. Gene mutations were induced in the *tk* locus in cells exposed to the chemical for 24 hours in the absence of metabolic activation (SCCP, 2008; REACH).

In vivo, negative results were obtained in the mammalian erythrocyte micronucleus test in male ICR mice (OECD TG 474) at doses 0, 37.5, 75, and 150 mg/kg bw (maximum tolerated dose); and in an unscheduled DNA synthesis test in male Sprague Dawley (SD) rats (OECD TG 486) at doses of 100 and 200 mg/kg bw (maximum tolerated dose) (SCCP, 2008; REACH). In both studies, the chemical was administered by oral gavage.

Carcinogenicity

No data are available.

Reproductive and Developmental Toxicity

The potential for the chemical to cause reproductive toxicity has been examined in rabbits and rats. Most of these studies were non-guideline compliant and were conducted prior to the establishment of GLP. Based on the available information, the chemical does not show specific reproductive or developmental toxicity. Reproductive and developmental effects were only observed secondary to maternal toxicity.

Reproductive toxicity

In a one generation reproductive toxicity investigation (non-guideline, pre-GLP), the chemical was tested in male (n=10) and female (n=20) Charles River rats in a 2-phase study (SCCP, 2005; REACH). In phase one, male rats were given 0, 7.2, 36, or 100 mg/kg bw/day of climbazole (suspended in 3.0 % aqueous CMC solution) via oral gavage, daily for 10 weeks prior to mating. The female rats received the same doses commencing 14 days prior to mating. Both males and females continued to receive treatment throughout the mating, gestation and lactation periods. To examine the effects of climbazole on the oestrus cycle of the female rats, vaginal smears were taken daily. Evaluation of the uterine content was conducted on gestation day 13 in half of the treated females, while the rest of the females were allowed to deliver. The resulting pups were counted, examined for weight and sex, and monitored regularly during lactation. The dams were also examined for external and internal abnormalities. Females that failed to deliver by the 24th day after mating were sacrificed (REACH).

Exposure of males to doses of climbazole at 7.2 and 36 mg/kg bw/day did not induce toxicity. At 100 mg/kg bw/day, the males showed hair loss, salivation, and increased activity. Similar effects were noted in females treated with 36 and 100 mg/kg bw/day climbazole, including death of one female in each dose group during delivery. Except for the increased activity in males which persisted through the study, these changes were observed less frequently over the course of the treatment. Observations reported in females at the 100 mg/kg bw/day group included: presence of red ocular and nasal discharge and urine stains in the abdomen; self-mutilation of the extremities; increased length of gestation; reduced fertility; and elongated stages of dioestrus. However, the study did not indicate whether these changes were statistically significant (REACH).

Compared with controls, effects observed in female rats at the 100 mg/kg bw/day dose group included: reduction in the mean number of viable and total implantations and the ratio of implantation sites to corpora lutea; increase in resorption; decrease in the number of pups born alive or the number of pups born; and significant decrease in the number of live pups per litter at birth (REACH).

Due to the effects of the chemical in female rats at the 100 mg/kg bw/day dose, phase two of this study was conducted to examine the effects of climbazole on male rat reproduction. In this phase, males were treated with identical doses of climbazole as described in phase one (see above) 86 days before mating and throughout the study period. Females did not receive treatment in this phase of the study. Compared with controls, no difference was noted in the general behaviour and appearance, survival or body weight gains in the parent rats and the litters. Climbazole did not affect the fertility of the male rats in phase two of the study.

The NOAEL values derived from this study are as follows: 7.2 mg/kg bw/day for maternal toxicity; 100 and 36 mg/kg bw/day for reproductive toxicity in males and females respectively (SCCP, 2005; REACH).

Developmental toxicity

In a non-guideline oral study in Charles River rats (SCCP, 2005), climbazole (doses: 0, 7.2, 36 or 100 mg/kg bw/day) was administered to 25 inseminated female rats daily by oral gavage from day six to day 15 of pregnancy. All dams were sacrificed on day 20 and foetuses were delivered through caesarean section. The effects of climbazole treatment on resorptions, total implantations and corpora lutea were evaluated. The body weights, sex ratios, and abdominal and thoracic cavities of the resulting foetuses were examined. Visceral malformations and skeletal deformation and variations were evaluated in some of the foetuses. At 100 mg/kg bw/day, death of four animals on gestation day 10 was reported. At 36 mg/kg bw/day, hair loss and self mutilation (scabbing of open wounds) of the extremities and abdominal area were seen in animals. These observations were also reported in the highest dose group with the following additional findings: transient increased activity; varying degree of red ocular and nasal discharge; anogenital staining; red vaginal discharge. As the treatment progressed, these effects decreased. Changes in body weights and body weight gains were noted in the first three days. Significant reduction of post implantation loss was observed in animals from the 36 mg/kg bw/day group only. Increased incidence in the number of foetuses and litters with presacral vertebrae, 14th rudimentary ribs, and 14th full ribs were also observed in the 36 and 100 mg/kg bw/day dose groups. Although foetal malformations were seen in all dose groups, these were not a consistent type. Thus, this was not considered to be treatment-related. The NOAEL for maternal and foetal toxicity was 7.2 mg/kg bw/day (SCCP, 2005; REACH).

In another non-guideline compliant study predating GLP conducted in BAY:FB_{30} rats (n=25), mated females received 0, 10, 30, 100 mg/kg bw/day of climbazole in 0.5 % aqueous Tylose solution, daily from day six to day 15 of pregnancy by oral gavage. The animals received a total of 10 treatments. The foetuses were delivered by Caesarian section on day 20 and examined for treatment-related effects on body weights, sex, external and visceral malformations and skeletal deformities. The chemical did not cause maternal or foetal toxicity up to 30 mg/kg bw/day. The effects were more prominent in females treated with 100 mg/kg bw/day of climbazole: 21/25 females appeared unhealthy; 8/25 self-mutilated; and the embryos in 8 animals were resorbed during pregnancy. In this dose group, effects on foetal sex ratio was also observed, with the percentage of male foetuses lower than females. Although an increase in foetal resorption was reported in the study, the chemical was not considered to be teratogenic in rats. This effect was considered a result of maternal toxicity (SCCP, 2005; REACH).

In a guideline compliant developmental toxicity study (OECD 414) conducted in Chinchilla rabbits (n=24/group) (SCCP, 2005), mated rabbits were given 0, 15, 30, or 60 mg/kg bw/day of climbazole (in 4 % CMC) daily by oral gavage from day six to day 27 post coitum. At day 28, the pregnant rabbits were sacrificed and the resulting foetuses were evaluated for the following: body weights, sex ratios, external and fresh visceral, fixed foetal heads, thoracic organs, skeleton and cartilage. Deaths were reported in one dam from the 30 mg/kg bw/day group at day 28 and one dam from the 60 mg/kg bw/day group at day 27. In the latter group, one animal was sacrificed in extremis (point of death) at day 25. The animals that spontaneously aborted (one animal/group) from 0, 15, and 30 mg/kg bw/day groups at days 26, 28, 26, respectively, were sacrificed thereafter. The abortions were not considered treatment-related due to the lack of observed dose-response pattern (SCCP, 2005). At the top two doses (30 and 60 mg/kg bw/day), the following maternal effects were observed: local hair loss (alopecia), and dose-related reduction of

food consumption at day six to day 18. Post implantation losses were reported in seven rabbits at 60 mg/kg bw/day, and one each from 30 mg/kg bw/day and control groups. Decreases in the number of foetuses were noted in animals from the 30 mg/kg bw/day group and this effect was significant at 60 mg/kg bw/day. Compared with controls, the proportion of female foetuses was higher at 30 mg/kg bw/day and was significantly higher at 60 mg/kg bw/day. Based on these results, the chemical is not considered to cause developmental toxicity, and the developmental effects seen were secondary to maternal toxicity. The maternal and foetal NOAEL values derived from this study were 15 and 60 mg/kg bw/day, respectively (SCCP, 2005; REACH).

Based on the results from the available low quality studies, the potential for climbazole to cause reproductive and developmental toxicity cannot be ruled out. The effects described in the previous sections have flagged some concerns in the EU. As a result, climbazole has been selected as candidate substance for the EU's Community Action Rolling Plan (CoRAP) initiative. Under this initiative, further evaluation will be conducted on the reproductive and developmental toxicity of climbazole.

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include acute toxicity from oral exposure. The chemical could also cause reproductive and/or developmental toxicity.

Public Risk Characterisation

Although use in cosmetic or domestic products in Australia is not known, the chemical is reported to be used in cosmetic products overseas.

There are existing restrictions on the use of the chemical in cosmetic products in Australia and overseas (see **Restrictions: International**). In the EU, the Scientific Committee on Consumer Safety (SCCS) concluded that there is a safety concern when the chemical is used as in body creams at 0.5 % or in a combination of three cosmetic products (shampoo, hair lotion, and face cream) containing the chemical at concentrations of 0.5-2.0 % for each product (SCCS, 2013). The calculated margins of safety were 13 (body cream) or approximately 100 (when used in combined products), based on a conservative NOAEL of 5 mg/kg bw/day chosen due to increased liver weights and enzyme changes at the next highest dose (SCCP, 2008).

Occupational Risk Characterisation

During product formulation, exposure may 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 local health effects, the chemicals could pose an unreasonable risk to workers unless adequate control measures to minimise dermal exposure are implemented. Hence, 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 HSIS (Safe Work Australia) (refer to **Recommendation** section).

NICNAS Recommendation

Further risk management is required. Sufficient information is available to recommend that risks to public health and safety from the potential use of the chemical in cosmetics and/or domestic products be managed through changes to poisons scheduling, and risks for workplace health and safety be managed through changes to classification and labelling.

Assessment of the chemical is considered to be sufficient provided that risk management recommendations are implemented and all requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory.

Regulatory Control

Public Health

It is recommended that an amendment to the current listing of the chemical in the SUSMP be considered. Given the risk characterisation, it is recommended that the concentration of the chemical in cosmetics and personal care products be restricted. Matters to be taken into consideration include:

- restrictions on using the chemical in cosmetics exist in the EU and various other countries (see **Restrictions: international** section); and
- bioavailability and dermal absorption profile of the chemical (see **Toxicokinetics** section).

Work Health and Safety

The chemical is recommended for classification and labelling under the current approved criteria and adopted GHS as below. This assessment does not consider classification of physical and environmental hazards.

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Acute Toxicity	Harmful if swallowed (Xn; R22)	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 consumers

Products containing the chemical should be used according to the instructions on the label.

Advice for industry

Control measures

Control measures to minimise the risk from oral, inhalation, dermal and ocular 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 chemicals are used. Examples of control measures which could minimise the risk include, but are not limited to:

- air monitoring to ensure control measures in place are working effectively and continue to do so;
- health monitoring for any worker who is at risk of exposure to the chemical[s], if valid techniques are available to monitor the effect on the worker's health;

- 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 these chemicals has not been undertaken as part of this assessment.

References

ChemIDPlus Advanced. Accessed December 2015 at http://chem.sis.nlm.nih.gov/chemidplus/

CosIng (Cosmetic Ingredients& Substances) Database. European Commission. Available: http://ec.europa.eu/consumers/cosmetics/cosing/

Galleria Chemica. Accessed January 2016 at http://jr.chemwatch.net/galleria/

Kobayashi Y, Ohshiro N, Sunagawa T, Oguro T, Tokuyama S, Yamamoto T, Yoshida T 2002. Induction and inhibition of cytochrome P450 and drug-metabolising enzymes by climbazole. Biological and Pharmaceutical Bulletin 25 pp. 53-57.

Personal Care Products Council (INCI Dictionary). Accessed January 2016 at http://www.ctfa-gov.org/jsp/gov/GovHomePage.jsp

Registration, Evaluation and Authorisation of Chemicals (REACH) Dossier. (REACH). Climbazole (CAS No. 38083-17-9). Accessed December 2015 at http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

Safe Work Australia (SWA). Hazardous Substances Information system (HSIS). Accessed December 2015 at http://hsis.safeworkaustralia.gov.au/HazardousSubstance

Scientific Committee on Consumer Products (SCCP) 2005. Opinion on Climbazole COLIPA No. P64. SCCP/1204/05. Adopted at its 5th plenary meeting of 20 September 2005. Accessed December 2015 at

http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_027.pdf

Scientific Committee on Consumer Products (SCCP) 2008. Opinion on Climbazole COLIPA No. P64. SCCP/1204/08. Adopted at its 19th plenary meeting of 21 January 2009. Accessed December 2015 at http://ec.europa.eu/health/archive/ph_risk/committees/04_sccp/docs/sccp_o_164.pdf

Scientific Committee on Consumer Safety (SCCS) 2013. Opinion on Climbazole regarding potential development of (cross)-resistance. Cosmetics Europe:P64. Adopted at its 18th plenary meeting of 26 February 2013. Accessed January 2016 at http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_121.pdf

The Poisons Standard (the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP)) 2015. Accessed December 2015 at https://www.comlaw.gov.au/Details/F2015L01534

United States Pharmacopeia (USP), 2011. Authorised USP Pending Monograph Version 1. Accessed January 2016 at http://www.usp.org/sites/default/files/usp_pdf/EN/USPNF/pendingStandards/m4127_authorized.pdf

van Meerloo J, Kaspers GJ, Cloos J 2011. Cell sensitivity assays: the MTT assay. Methods in Molecular Biology 731 pp. 237-245.

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