9H-Carbazole: Human health tier II assessment

30 June 2017

CAS Number: 86-74-8

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.

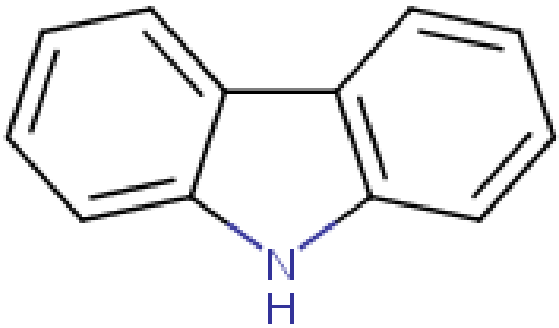
This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

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

Disclaimer

Acronyms & Abbreviations

Chemical Identity

Synonyms	carbazole 9-azafluorene diphenylenimine dibenzopyrrole
Structural Formula	
Molecular Formula	C12H9N
Molecular Weight (g/mol)	167.21
Appearance and Odour (where available)	White or tan powder.
SMILES	c12-c3c(cccc3)Nc1cccc2

Import, Manufacture and Use

Australian

No specific Australian 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 Substances and Preparations in Nordic countries (SPIN) database; the United States (US) Personal Care Products Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary; the Organisation for Economic Cooperation and Development High Production Volume chemical program (OECD HPV); the US Environmental Protection Agency's Aggregated Computer Toxicology Resource (ACToR); and the US National Library of Medicine's Hazardous Substances Data Bank (HSDB).

The chemical has reported site-limited uses, including as an intermediate for manufacturing dyes, pigments, lubricants, rubber antioxidants and photographic plates sensitive to ultraviolet light.

The chemical has a reported domestic use, including as a component in detergents. However, no evidence of the presence of the chemical in consumer products was found in available North American databases (Household Products Database), indicating that the chemical is not likely to be widely available for domestic uses.

The chemical has reported non-industrial uses as an intermediate for manufacturing insecticides and explosives.

Restrictions

Australian

No known restrictions have been identified.

International

No known restrictions have been identified.

Existing Work Health and Safety Controls

Hazard Classification

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

Exposure Standards

Australian

No specific exposure standards are available.

International

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

Temporary Emergency Exposure Limits (TEELs) defined by the US Department of Energy (DOE):

TEEL-1 = 0.66 mg/m^3

TEEL-2 = 7.2 mg/m^3

TEEL-3 = 43 mg/m^3

Health Hazard Information

Toxicokinetics

Limited data are available on the toxicokinetics of the chemical.

Administration of ^{14}C-radiolabelled carbazole at single doses of 4 mg/kg bw in mice (n=6, by intraperitoneal (i.p.) injection) and 1000 mg in a rabbit (by oral administration) showed that the chemical was glucuronised and excreted in the urine. In rats, approximately 65 % of the radiolabelled chemical was excreted in the urine within 3 days. The chemical was observed to have low bioaccumulation potential. The major metabolite identified in rats and the single rabbit was 3-hydroxycarbazole (Johns and Wright, 1964; REACH).

No other toxicokinetic information is available.

Acute Toxicity

Oral

The chemical has low acute toxicity based on results from animal tests following oral exposure. The median lethal dose (LD50) in rats was not determined in the available guideline tests since no mortality was observed at the maximum dose of 16000 mg/kg bw. Observed sub-lethal effects included unkempt hair coat, contorted posture, pallor of extremities, lacrimation, diarrhoea, mid to high grade lethargy and ataxia.

In an acute study similar to the OECD Test Guideline (TG) 401 (Acute Oral Toxicity), Wistar rats (5/sex) received single doses of 16000 mg/kg bw of the chemical suspension (40 % carbazole in 0.5 % carboxymethyl cellulose) by gavage. No mortality was reported within the observation period of 14 days. The target organs were reported to be the lungs and liver (REACH).

Other studies have reported lowest lethal doses (LDLo) in rats ranging from 500 to 5000 mg/kg bw (Galleria Chemica). These studies have provided limited information on the study methods.

Dermal

No data are available.

Inhalation

No data are available.

Corrosion / Irritation

Skin Irritation

The chemical is reported to be a slight skin irritant in animal studies. The effects were not sufficient to warrant hazard classification.

In a skin irritation study conducted in accordance with the US Consumer Product Safety Commission, Code of Federal Regulations, 5 mL of a 10 % solution of the chemical (in 0.5 % carboxymethyl cellulose) was applied on intact and scarified shaved backs of albino rabbits (6 animals; unspecified sex) under occlusive conditions for 24 hours. The observation period was 72 hours after application. Minimal erythema and oedema were observed on the intact skins of three animals immediately after removal of the patch. Effects were reversible within 48 hours. Irritation effects observed on scarified skin were fully reversible within 72 hours (REACH).

In another study, the chemical (5 % solution in olive oil) was applied (20 applications) to the ears and shaved bellies of rabbits (unspecified species) for 28 days. Mild irritations, characterised by very slight hyperaemia and scaliness, were reported. No gross or microscopic changes were observed (Anon, 1988).

In a phototoxicity assay conducted in Hartley guinea pigs (6 animals), increasing doses of the chemical (0.836, 8.36, 83.6 and 836 mg/L) were applied on the clipped and depilated skin of each guinea pig followed by exposure to UV-A radiation (30 minutes prior to UV radiation, 40 to 80 minutes irradiation, 20 hours post-irradiation). Scores were obtained 20 hours after irradiation. There was no irritation reported in the absence of UV light and there was no phototoxicity in the presence of UV light observed (REACH).

Eye Irritation

The chemical is reported to be a slight eye irritant in an animal study. The effects were not sufficient to warrant hazard classification.

The chemical was reported as ‘not irritating’ after a 10 % suspension of carbazole solution (in 0.5 % carboxymethyl cellulose) was instilled to the eyes of six albino rabbits. Observation times were 24, 48, 72 hours, and seven days following treatment. Mild chemosis, which fully reversed within one day, was reported. However, redness of the conjunctivae did not completely disappear at day 7 (REACH).

Sensitisation

Skin Sensitisation

No data are available.

Repeated Dose Toxicity

Oral

Limited data are available on the repeat dose toxicity of the chemical. The available data did not warrant hazard classification of the chemical.

In mice (unspecified number, strain and sex), a lowest toxic dose (TDLo) of 25200 mg/kg bw was reported for the chemical following 12 weeks of oral administration (Galleria Chemica). The most common systemic effects reported included changes in liver weight, body weight loss or decreased weight gain. No other information was provided. It is unclear whether the dose provided was the daily dose or the total dose over 12 weeks, although in the worst case the total dose would be >250 mg/kg bw/day.

Dermal

Only limited data are available on the repeat dose toxicity of the chemical.

A solution of 0.5 % carbazole in benzene was applied to the skin of 50 mice (unspecified number, strain and sex) for 120 applications. Epilation of the treated area was the only reaction observed after 276 days (IARC, 1983).

Inhalation

No data are available.

Genotoxicity

Based on the weight of evidence from the in vitro and in vivo genotoxicity studies that were conducted in accordance with the OECD TGs, the chemical is considered to be genotoxic, warranting classification (see **Recommendation** section). Available in vitro genotoxicity tests indicated negative results, but the in vivo tests, including a Rodent Dominant Lethal assay, were positive.

The chemical has been reported to be negative in in vitro mutagenicity tests, including:

- Bacterial mutation assays using *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and/or TA100 (IARC, 1983; 1999; REACH), and in tests using wild or DNA-repair deficient strains of *Bacillus subtilis* (Anon, 1988).
- A study using rat hepatocytes conducted according to the OECD TG 482 (Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells In Vitro) when carbazole was tested at concentrations ranging from 0.167 to 167 mg/L (REACH).

However, in vivo studies conducted according to the OECD TGs combined provide conclusive evidence demonstrating the genotoxic properties of the chemical. The difference observed between in vitro and in vivo studies may be related to different metabolic processes.

Two in vivo cell mutagenicity assays of the chemical gave positive results (REACH):

- A Mammalian Bone Marrow Chromosomal Test (equivalent to OECD TG 475) was performed on Swiss albino mice to evaluate the clastogenicity of carbazole. The chemical was administered at i.p. doses of 25, 50, 100, 150 and 200 mg/kg bw. The mice were euthanised at 14 and 42 hours after treatment. Dose-dependent mitotic depression after treatment was reported for both time points. However, statistically significant reduction of mitotic index (compared to solvent control) and an increase in chromosomal aberrations per cell were only observed at the two highest doses (Jha et al., 2002). Based on this study, the chemical is considered to be moderately clastogenic in the bone marrow cells of mice in vivo under the test conditions used (REACH).
- A Rodent Dominant Lethal Test (equivalent to OECD TG 478) was performed in Swiss albino male mice (20 per group). The chemical (at i.p. doses of 30 and 60 mg/kg bw/day) was injected for five consecutive days. Treatment resulted in appreciably higher percentage of dominant lethal mutation as compared to solvent (dimethyl sulfoxide (DMSO)) control (Jha and Bharti, 2002).

Carcinogenicity

Based on the available data below, the chemical may have possible carcinogenic activity in animals, warranting hazard classification (see **Recommendation** section).

The International Agency for Research on Cancer (IARC) has classified the chemical as 'possibly carcinogenic to humans' (Group 2B), based on 'limited evidence of carcinogenicity in humans and less than sufficient evidence of carcinogenicity in experimental animals' (IARC, 2013).

In a carcinogenicity study according to OECD TG 451, the chemical was fed to B6C3F$_1$ mice (n= 50/sex) at concentrations of 0, 0.15, 0.3 or 0.6 % in diet for 96 weeks, followed by eight weeks of basal diet. Statistically significant increases in the incidences of neoplastic lesions in the liver and forestomach were reported in all treatment groups. All treated groups showed increased incidence of pulmonary metastases (Tsuda et al., 1982).

Several other in vivo studies have investigated the carcinogenicity effects of carbazole in conjunction with other chemicals. Although the reliability of these studies is questionable, as the methods used were not entirely in accordance with approved guidelines on carcinogenicity studies, the results support the classification of carbazole as a suspected human carcinogen.

In a promotion study, male Fischer 344 (F344) rats were given drinking water that contained 0.2 % of the initiator, N-bis(2-hydroxypropyl) nitrosamine (DHPN), for one week, followed by a diet containing 0.6 % carbazole for 50 weeks. Control animals were treated with DHPN or carbazole alone. Groups treated with both DHPN and carbazole resulted in higher incidence of papillomas of the renal pelvis and tumours of the urinary bladder (papillomas or carcinomas), when compared to treatment with DHPN alone. These effects suggest that the chemical promotes the induction of renal pelvic tumours (Shirai et al., 1988).

In a separate study, F344 rats were given drinking water containing 0.05 % N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) for 2 weeks, followed by a diet containing 0.6 % carbazole for 22 weeks. The results indicated that treatment with 0.6 % carbazole significantly increased the incidence of papillary and nodular hyperplasia, but not urinary bladder carcinogenesis (Miyata et al., 1985). Another study investigated the effect of carbazole on the development of preneoplastic lesions in Syrian golden hamsters. The chemical was administered in the diet at 0.2 % concentration either alone or subsequent to a subcutaneous injection of 2,2'-dioxo-N-nitrosodipropylamine (DOPN; 20 mg/kg bw). Treatment with carbazole significantly increased the number and size of hepatocellular foci in both treatment groups (with or without prior injection with DOPN) (Moore et al., 1987).

Reproductive and Developmental Toxicity

Based on the limited information available, the chemical does not show specific reproductive or developmental toxicity.

In a developmental toxicity study conducted according to the US Environmental Protection Agency (Prenatal Developmental Toxicity) TG, six groups of 12 pregnant Sprague Dawley (SD) rats were topically applied carbazole (in DMSO) at doses of 2.5, 25, 250 mg/kg bw/day on gestation days (GD) 0 through to 20. A separate group of 15 pregnant SD rats served as vehicle controls and were treated with DMSO. Dams were allowed to deliver and all animals including pups were euthanised at lactation day 4. Dams were monitored for signs of toxicity throughout gestation. Pups were weighed after birth and at day 4 of lactation. Gross abnormalities and mortality of each litter was determined on day 1 and 3 of lactation. The study concluded that carbazole did not induce maternal or developmental toxicity in rats up to the highest concentration tested (250 mg/kg bw/day) (Dutson et al., 1997).

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include systemic long-term effects (carcinogenicity and mutagenicity).

Public Risk Characterisation

Given the uses identified for the chemical, it is unlikely that the public will be exposed. Hence, the public risk from this chemical is not considered to be unreasonable.

Occupational Risk Characterisation

Given the critical systemic long-term health effects, the chemical could pose an unreasonable risk to workers unless adequate control measures to minimise exposure are implemented. The chemical 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 chemical is 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.

Regulatory Control

Work Health and Safety

The chemical is 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]
Genotoxicity	Not Applicable	May cause genetic defects - Cat. 1B (H340)
Carcinogenicity	Not Applicable	Suspected of causing cancer - Cat. 2 (H351)

[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, dermal and inhalation exposure to the chemical 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:

- using closed systems or isolating operations;
- using local exhaust ventilation to prevent the chemical from entering the breathing zone of any worker;

- health monitoring for any worker who is at risk of exposure to the chemicals, if valid techniques are available to monitor the effect on the worker's health;
- air monitoring to ensure control measures in place are working effectively and continue to do so;
- minimising manual processes and work tasks through automating processes;
- 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 chemical.

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 chemical 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 chemical has not been undertaken as part of this assessment.

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Last update 30 June 2017

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