Butanamide, 2-[(2-methoxy-4-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxo-: Human health tier II assessment

13 February 2015

CAS Number: 6358-31-2

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.

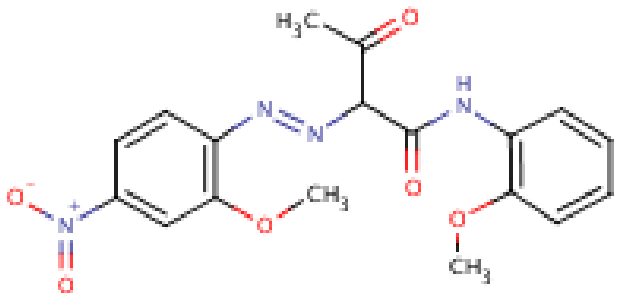
This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

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

Disclaimer

Acronyms & Abbreviations

Chemical Identity

Synonyms	C.I. Pigment Yellow 74 Lunar yellow 2-((2-Methoxy-4-nitrophenyl)azo)-N-(2-methoxyphenyl)-3-oxobutyramide
Structural Formula	
Molecular Formula	C18H18N4O6
Molecular Weight (g/mol)	386.4
Appearance and Odour (where available)	Solid bright yellow powder with a non-specific odour.
SMILES	c1(N=NC(C(C)=O)C(=O)Nc2c(OC)cccc2)c(OC)cc(

	N(=O)=O)cc1

Import, Manufacture and Use

Australian

The following Australian industrial uses have been identified from safety data sheets (SDS).

The chemical has reported domestic uses including in:

- wood and metal marking materials; and
- paints.

International

The following international uses have been identified through:

- European Union (EU) Registration, Evaluation and Authorisation of Chemicals (REACH) dossiers;
- Galleria Chemica; the Substances and Preparations in the Nordic countries (SPIN) database;
- the United States (US) National Library of Medicine's Hazardous Substances Data Bank (HSDB); and
- the Danish Ministry of the Environment (Danish EPA, 2012).

The chemical has reported cosmetic use in tattoo inks.

The chemical has reported domestic uses including in:

- corrosion inhibitors;
- surface treatments;
- adhesives, binding agents;
- paints, lacquers, varnishes; and
- pigments, colourants, dyes and printing inks.

The chemical has reported commercial uses including:

- in construction materials;
- in polymer preparations and compounds;
- as reprographic agents; and
- in leather tanning.

The chemical has reported non-industrial use in non-agricultural pesticides and preservatives.

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 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

Butanamide, 2-[(2-methoxy-4-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxo-, also known as Colour Index (C.I.) Pigment Yellow 74, and referred to as such henceforth, is an azo compound typically used in dyes, colourants and pigments. The chemical has reported use in cosmetic applications as a tattoo ink (Cul et al., 2004).

Toxicokinetics

Few studies have assessed the toxicokinetics of C.I. Pigment Yellow 74. The available data are summarised below (REACH):

Absorption, distribution and excretion

In a 1984 study that used male Fischer 344 (F344) rats to assess toxicokinetics, no evidence of systemic absorption of the chemical was observed following administration of a single dose by gavage at 12.6 mg/kg body weight (bw). Small quantities of the compound were present in tissues that were in direct contact with the chemical; however, this was attributed to mechanical adherence, rather than absorption. At one and 24 hours after dosing, the majority of the chemical was present in the gut contents and none in the faeces. At four and 48 hours after dosing, the majority of the compound was found in the faeces (up to 86 %). Small amounts (<3 %) were found in tissues that were in direct contact with the test material. The chemical was not found in whole blood, the liver, kidneys or lungs, even following administration of doses 10 times larger. Investigators also reported that approximately 86 % of the administered test material was excreted via the faeces after 48 hours.

Metabolism

The metabolism of C.I. Pigment Yellow 74 was assessed in a 2005 study in an in vitro test system consisting of rat and human liver microsomes, expressing cytochromes P450 (CYPs). Metabolism of the test material resulted in two distinct metabolites: a ring hydroxylation product and 2-((2-hydroxy-4-nitrophenyl)azo)-*N*-(2-methoxy-4-hydroxyphenyl)-3-oxobutanamide, which is the *O*-demethylation product of C.I. Pigment Yellow 74. The enzyme CYP 1A2 had the highest activity, indicating that it is the key protein responsible for metabolism of the chemical (REACH).

Azo bonds can be reduced by azo reducatases and also photolytically (Cul et al., 2004; Bafana et al., 2011). C.I. Pigment Yellow 74 will produce the carcinogenic amine anisidine (NICNAS) on reduction of one azo bond.

Acute Toxicity

Oral

The chemical has low acute toxicity based on findings from animal tests following oral dosing. The median lethal dose (LD50) in rats is >2000 mg/kg bw. No sub-lethal effects were observed.

A study was conducted to assess the acute toxicity of C.I. Pigment Yellow 74 in accordance with the Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 401 (acute oral toxicity). Wistar rats of both sexes were administered a single dose of the test material by oral gavage at a concentration of 2000 mg/kg bw. No abnormalities or gross pathological changes were observed in any of the test animals and no mortalities were recorded. The chemical was detected in the faeces of all rats two and three days following administration, but was not detected from the fourth day onwards. Under these test conditions, the oral LD50 of C.I. Pigment Yellow 74 was found to be >2000 mg/kg bw (REACH).

In another study, performed under conditions similar or equivalent to OECD TG 401, a single oral dose of the test material at a concentration of 15000 mg/kg bw was not lethal in male Wistar rats. No sub-lethal effects were observed in these animals (REACH).

Dermal

No data are available.

Inhalation

No data are available.

Corrosion / Irritation

Skin Irritation

In studies that were performed in accordance with OECD TG 404 (acute dermal irritation/corrosion), the chemical did not cause skin irritation.

An OECD TG 404 study was performed to determine the corrosivity/irritancy of C.I. Pigment Yellow 74. The chemical (0.5 g) was mixed with paraffin oil and applied to the skin of New Zealand White rabbits under semi-occlusive patches and left for three, 60 or 240 minutes. Clinical assessments of animals were performed at one, 24, 48 and 72 hours post application. No skin reactions or pathological signs were observed in any animals at any time point and no pre-terminal mortalities were recorded. Under the test conditions, the chemical did not act as an irritant (REACH).

In another study, conducted in a similar fashion to OECD TG 404, the chemical (0.5 g) was applied occlusively on shaved intact and abraded portions of rabbit skin (strain and specific methodology not stated) for 24 hours. Animals were assessed clinically 24 and 72 hours after administration of the chemical and were observed for up to 14 days. No signs of skin irritation were

observed at any time point (erythema and oedema scores of 0 were recorded for all assessments). Under the test conditions, the chemical was found not to cause any skin irritation (REACH).

Eye Irritation

The chemical was reported to slightly irritate the eyes when tested according to OECD TG 405 (acute eye irritation/corrosion). The observed effects do not warrant hazard classification.

An OECD TG 405 study was conducted to determine the potential of the chemical to cause ocular irritation. A volume of 0.1 mL (14 mg) of the chemical was instilled in one eye of three New Zealand White rabbits. Clinical assessments were performed at 24, 48 and 72 hours after exposure. The authors reported that one animal exhibited grade 1 conjunctival erythema at the 24-hour time point under the Draize scoring system. All other readings of conjunctival erythema, chemosis, discharge, iris and cornea effects in all three animals, at all time points, were assigned grade 0. No pre-terminal mortalities occurred and no abnormal necropsy findings were reported. Under the test conditions, the chemical caused only mild, transient signs of eye irritation (REACH).

Another study was conducted similarly to OECD TG 405 to assess the chemical as an ocular irritant. The eyes of rabbits were exposed to 0.1 g of the chemical and were clinically assessed one, 24, 48, 72 and 144 hours following exposure. This study demonstrated that the test material induced only mild, transient signs of eye irritation (REACH).

Sensitisation

Skin Sensitisation

The chemical was not found to induce dermal sensitisation when tested according to OECD TG 429 (skin sensitisation—local lymph node assay).

A recent study investigated the sensitisation potential of the chemical in accordance with OECD TG 429. Four female CBA mice were treated with the chemical at 0, 2.5, 5 and 10 % (w/v) in suspension. The animals did not show any clinical signs during the course of the study and no cases of mortality were observed. In this study, stimulation index (SI) values of 0.45, 1.02, and 2.18 were determined with the test item at concentrations of 2.5, 5, and 10 %, respectively. Under the conditions of this study, no EC3 value (estimated concentration to achieve an SI of 3) could be determined and the chemical was not considered to be a skin sensitiser (REACH).

Another skin sensitisation study was conducted in accordance with OECD TG 429. In this local lymph node assay, female CBA mice were topically administered the chemical at 0, 1, 2.5 and 5 % (w/v) in a mixture of acetone and olive oil. Mean SI values of 0.7, 0.6 and 1.4 were determined for the concentrations of 1, 2.5 and 5 %, respectively. Under the test conditions, the chemical was found not to act as a skin sensitiser (REACH).

Repeated Dose Toxicity

Oral

In a 90-day oral gavage study in rats, a no observed adverse effect level (NOAEL) of 1000 mg/kg bw/day for the chemical was reported.

A study conducted in compliance with OECD TG 408 (repeated dose 90-day oral toxicity in rodents) assessed the repeated dose toxicity of the chemical in F344/DuCrl rats. Following a preliminary range-finding experiment, the chemical was administered to treatment groups of animals daily by gavage at 0, 50, 200 or 1000 mg/kg bw/day. Numerous physiological and pathological parameters were assessed in this study including clinical signs, ophthalmoscopy, body weight, haematology, feed consumption, urinalysis, necropsy, organ weights and histopathology.

The chemical caused a dose-dependent increase in female liver weights. However, the absence of any other significant histopathological or haematological change in test animals suggested the liver change was more likely a result of an adaptive, rather than an adverse, effect. No other test substance-related effects were noted. Therefore, under the conditions of this study, the NOAEL was established at 1000 mg/kg bw/day in both sexes.

Another study assessed the repeated dose toxicity of the chemical in a non-guideline study. Mixed race albino rats (strain not stated) of both sexes (5/sex) were administered the chemical by oral gavage at 500 mg/kg bw/day, 14 times over a period of 18 days. Body weights of animals were assessed weekly, and blood (haemoglobin content, erythrocytes, leukocytes) and urine (appearance, colour, protein, sediment) analysis were performed at the start and end of the study. Following the test period, animals were euthanised and macroscopic and histological assessments of the heart, lungs, liver, kidneys and spleen were performed. No effects of repeated exposure to the chemical were observed. Therefore, under these test conditions, the chemical had a NOAEL of 500 mg/kg bw/day (REACH).

Dermal

No data are available.

Inhalation

No data are available.

Genotoxicity

Considering the negative in vitro genotoxicity data and the lack of systemic absorption observed in the toxicokinetic studies, the chemical is not expected to be genotoxic. However, the potential breakdown product, anisidine, is positive for genotoxicity (NICNAS).

In vitro

A study was performed in compliance with OECD TG 471 (bacterial reverse mutation assay), to assess the genotoxicity of the chemical, using *Salmonella typhimurium* TA 1535, TA 1537, TA 98 and TA 100 strains as well as *Escherichia coli* WP2 uvrA. The chemical was tested at 0, 3, 10, 33, 100, 333, 1000, 2500 or 5000 µg/plate in the presence or absence of metabolic activation. No significant increase in the number of revertant colonies was observed in the five bacterial test strains assessed, at any concentration, with or without metabolic activation. Therefore, under the test conditions, the chemical failed to induce frameshift or point mutations and, as a result, is not considered to be genotoxic.

Another study, conducted in compliance with OECD TG draft proposal 487 (in vitro micronucleus test), assessed the genotoxicity of the chemical. Chinese hamster lung fibroblasts (V79) were treated with the chemical for four or 24 hours at concentrations of 1.2, 2.4, 4.7, 9.4, 18.8, 37.5, 75.0, 150.0, 300.0, 600.0 or 1200.0 µg/mL both in the presence or absence of metabolic activation, across three separate experiments. The chemical did not induce micronuclei formation, an indicator of chromosomal damage, at any concentration in V79 cells. Therefore, under these experimental conditions, the chemical was not considered to be genotoxic (REACH).

An older non-guideline study assessed the genotoxicity of the chemical in a mammalian gene mutation assay. Mouse lymphoma L5178Y cells were treated with the test material at 0, 972, 1231, 1488, 1744 or 2000 µg/mL in the presence and absence of metabolic activation. The relative cloning efficiency, growth as well as mutant frequency, were not affected by the chemical. Therefore, under the test conditions, the chemical was not considered to be genotoxic.

The chemical has not been assessed in any in vivo genotoxicity studies.

Carcinogenicity

No data are available. However, given that the chemical has been shown not to exhibit genotoxicty, and considering the lack of systemic absorption observed in the toxicokinetic studies, the chemical is not anticipated to be carcinogenic, although conditions

where the azo bonds are reduced results in formation of anisidine, which is a probable human carcinogen (NICNAS; Stiborova et al., 2005).

Reproductive and Developmental Toxicity

No data are available. However, given that the chemical did not cause adverse effects on the reproductive organs in the repeated dose toxicity studies, and considering the lack of systemic absorption observed in the toxicokinetic studies, the chemical is not anticipated to exhibit specific reproductive or developmental toxicity.

Risk Characterisation

Critical Health Effects

No critical health effects have been identified, except under conditions where the azo bond is reduced.

Public Risk Characterisation

Although use in cosmetic products and tattoo inks in Australia is not known, the chemical is reported to be used in cosmetic products and tattoo inks overseas. Given their limited bioavailability, this chemical is not considered to pose an unreasonable risk to public health for the majority of cosmetic applications.

However, the intradermal injection of this chemical as an ink in tattoos is likely to be a potential health hazard. The decomposition or breakdown of this pigment in tattoo inks following exposure to a variety of light sources, such as lasers during tattoo removals, natural sunlight, and ultraviolet light might result in the compound being reduced into the carcinogenic aromatic amine anisidine (see **Toxicokinetics** section).

Considering anisidine is reasonably anticipated to be a human carcinogen (HSIS), the chemicals could pose an unreasonable risks to consumers if used in tattoo inks and permanent make-up.

A Tier III assessment to further investigate the risk of photodegradation of this pigment should be undertaken.

Occupational Risk Characterisation

During product formulation, ocular 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 chemical at lower concentrations could also occur while using formulated products containing the chemical. The level and route of exposure will vary depending on the method of application and work practices employed.

Given that critical health effects were not identified, an unreasonable risk to workers is not expected.

NICNAS Recommendation

Given the limited information on the photodegradation of pigments in tattoo inks and permanent makeup into carcinogenic substances, the chemical is recommended for Tier III assessment.

Formulators and importers of tattoo inks should consider substituting alternative products for items that contain this pigment.

All other risks are considered to have been sufficiently assessed at the Tier II level, subject to implementing any risk management recommendations, and provided that all requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory. The chemical is not recommended at this stage for classification and

labelling under the current approved criteria and adopted GHS. This assessment does not consider classification of physical hazards and environmental hazards.

Regulatory Control

Public Health

The need for further regulatory control for public health will be determined as part of the Tier III assessment.

Advice for industry

Control measures

Control measures to minimise the risk from oral and ocular 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 which could minimise the risk include, but are not limited to:

- using closed systems or isolating operations;
- 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 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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