Ethanol, 2-[(4-methyl-2-nitrophenyl)amino]-: Human health tier III assessment

25 November 2016

CAS Number: 100418-33-5

- Preface
- Synopsis
- Rationale for Tier III Assessment
- Chemical Identity
- Health Hazard Information
- Risk Characterisation
- NICNAS Recommendation
- References

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 III because the Tier II assessment indicated that it needed further investigation. The report should be read in conjunction with the Tier II assessment.

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

Disclaimer

Acronyms & Abbreviations

Synopsis

Under the Inventory Multi-tiered Assessment and Prioritisation (IMAP) Framework, it was determined that more work is required to assess the extent of use and determine appropriate risk management measures for some hair dye chemicals with limited hazard data. These chemicals were listed as being used in hair dyes in Australia in 2007 (NICNAS, 2007). The Tier II assessment report for these hair dye chemicals is available at: http://www.nicnas.gov.au/chemical-information/imap-assessments/imap-group-assessment-report?assessment_id=1702

This Tier III assessment focuses on the chemical methylgelb (CAS No. 100418-33-5),which was identified as being used in hair dyes in Australia following stakeholder consultations in 2015.

Rationale for Tier III Assessment

The chemical is listed on the 'List of chemicals used as dyes in permanent and semi-permanent hair dyes in Australia' (NICNAS, 2007). Following consultation with industry representatives in 2015, the chemical methylgelb (CAS No. 100418-33-5) has been confirmed to be used in hair dyes in Australia.

The use of the chemical in hair dyes is currently restricted in the European Union (EU) under the EU Cosmetics Regulation 1223/2009 Annex III - List of substances which cosmetic products must not contain except subject to the restrictions laid down. The maximum concentration allowed to be used in oxidative hair dyes is 2 % in ready to use preparations and 1 % upon application (CosIng). For non-oxidative hair dyes, the maximum concentration allowed in ready to use preparations is 1 %, but the CosIng indicates 'Not to be used after 31.12.2009'.

The use concentration of this chemical in hair dyes in Australia is not available to NICNAS. Currently, there are no concentration restrictions in Australia for using this chemical in hair dyes.

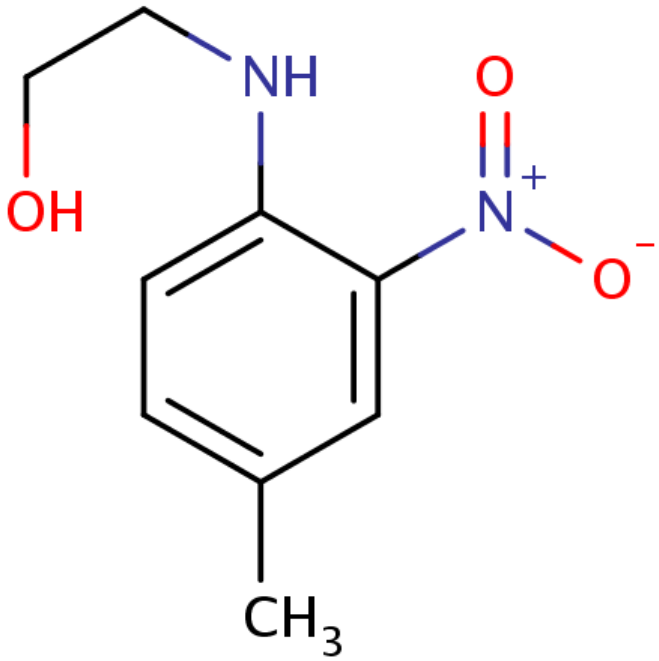
The chemical is classified as hazardous, with the following risk phrases for human health in the Hazardous Substance Information System (HSIS) (Safe Work Australia):

- Xn; R22 (acute toxicity); and
- Xi; R43 (sensitisation).

This Tier III assessment was conducted to determine if a risk management recommendation for public health is required for safe use of the chemical in hair dyes.

Chemical Identity

Synonyms	2-(4-methyl-2-nitroanilino)ethanol 4-((2-hydroxyethyl)amino)-3-nitro-1-methylbenzene hydroxyethyl-2-nitro-p-toluidine 2-((4-methyl-2-nitrophenyl)amino)ethanol methylgelb (trade name)
Structural Formula	



Molecular Formula	C9H12N203
Molecular Weight (g/mol)	196.2048
Appearance and Odour (where available)	Red crystalline powder
SMILES	c1(NCCO)c(N(=O)=O)cc(C)cc1

Health Hazard Information

All publicly available health hazard data on the chemical, mainly from the Scientific Committee on Consumer Safety (SCCS) report (2011), were used in this assessment.

Toxicokinetics

The dermal absorption of the chemical in hair dye formulations was investigated in vitro and in vivo. All studies showed that the chemical, up to 1 % final concentration in typical hair dye formulations, has low dermal absorption through human skin under oxidative and non-oxidative conditions.

In an in vitro percutaneous study compliant with the Organisation for Economic Cooperation and Development (OECD) Test Guideline (TG) 428, a hair dye formulation (non-oxidative) containing the chemical at 1 % concentration was tested using human skin samples from four donors. Results showed that an average amount of 1.55 % (3.08 µg/cm2) of the applied dose passed through the skin and was recovered in the receptor fluid within 72 hours post-exposure. The majority of the applied dose (95 %) was rinsed off the skin surface (SCCS, 2011).

In a second experiment following OECD TG 428, the chemical at 1 % concentration in an oxidative hair dye formulation was tested on human skin samples from four donors. A mean amount of 2.14 % (3.96 µg/cm2) of the applied dose passed through the skin and was recovered in the receptor fluid within 72 hours post-exposure. The majority of the applied dose (96 %) was rinsed off the skin surface (SCCS, 2011).

In an in vivo study (non-guideline), radiolabelled chemical in three formulations was applied onto the skin of Sprague Dawley (SD) rats (n = 3/sex/dose) for 30 minutes: two hair dye formulations containing the chemical at 1 % (without hydrogen peroxide) or 0.5 % (with hydrogen peroxide) and the chemical at 3.33 % in dimethyl sulfoxide (DMSO)/water. The majority of the applied dose (97–99 %) did not pass through the skin. The mean absorption was 0.21 and 0.24 % for the two hair dye formulations, and 0.69 % for DMSO/water. Most of the absorbed dose was excreted in the urine (80–85 %) and faeces (14–19 %), within 24 hours. Highest concentrations of 14C were detected in fat tissues, thyroid, liver, skin and kidneys, but there was no accumulation of the chemical as levels were below or near detection limits 72 hours post-application (SCCS, 2011).

Acute Toxicity

Oral

The chemical is classified as hazardous with the risk phrase ‘Harmful if swallowed’ (Xn; R22) in the HSIS (Safe Work Australia). The available data in rats and mice (median lethal dose (LD50) <2000 mg/kg bw) support this classification.

In a non-guideline acute oral toxicity study, Wistar rats (n = 6/sex/dose) and CF1 mice (n = 10/sex/dose) were administered the chemical as a single oral dose of 900, 1700 or 2500 mg/kg bw in rats and 1000, 1500, 2000 or 2500 mg/kg bw in mice. The oral LD50s were reported to be 1564 mg/kg bw in male rats, 1436 mg/kg bw in female rats, 1600 mg/kg bw in male mice and 1750 mg/kg bw in female mice. Signs of toxicity included reduced activity in both species (SCCS, 2011).

Dermal

The chemical haslow acute dermal toxicity. The dermal LD50in rats is >2000 mg/kg bw.

In an acute dermal toxicity study (OECD TG 402) in SD rats (n = 5/sex), the chemical was administered onto the skin as a single dose of 2000 mg/kg bw for 24 hours. No mortalities were observed. Observed sublethal effects included general distress, decreased body weights, ruffled fur and chromodacryorrhoea (red coloured tears) (SCCS, 2011).

Inhalation

No data are available.

Corrosion / Irritation

Skin Irritation

The chemical is not a skin irritant.

The chemical (0.5 g) applied as an occlusive patch to intact dorsal skin of three New Zealand White (NZW) rabbits (OECD TG 405) for four hours produced no skin irritation at 1, 24, 48 and 72 hours after patch removal (scores not available) (SCCS, 2011).

Eye Irritation

The chemical is not an eye irritant.

In a primary eye irritation study (OECD TG 405), the undiluted chemical (0.1 mL) was instilled into the left eye of three female NZW rabbits. Apart from minimal oedema of the conjunctivae in one rabbit (at 1-h observation) and redness of the conjunctivae observed in another rabbit (at 24-h observation), no other irritant effects were observed at 1, 24, 48, 78 and 168 h after application (SCCS, 2011).

Sensitisation

Skin Sensitisation

The chemical is classified as hazardous with the risk phrase ‘May cause sensitisation by skin contact’ (R43) in the HSIS (Safe Work Australia). The available data indicate that the chemical is not a skin sensitiser up to 10 % concentration. As there are no data to indicate that the concentrations above 10 % are not skin sensitising, removal of the existing hazard classification is not recommended.

In a local lymph node assay (LLNA) performed in accordance with OECD TG 429, groups of female CBA mice (n = 5/dose) were exposed to the chemical at 0, 0.5, 1.5, 5 or 10 % concentrations by application to the ear for three consecutive days. The chemical was dissolved in either dimethylsulfoxide (DMSO) or a mixture of water/acetone (1:1) with olive oil (3:1). The stimulation index (SI) was between 1.0–1.3 for all concentrations tested, indicating no sensitising effect (SI <3) up to the highest concentration tested (10 %) (SCCS, 2011).

Based on the results of this study, the SCCS (2011) concluded: 'The highest concentration tested (10%) was too low for hazard identification. Therefore, a sensitising potential cannot be excluded'.

Skin sensitisation prediction using OASIS–TIMES (Optimized Approach based on Structural Indices Set–TIssue MEtabolism Simulator) modelling was negative for the parent chemical, although the model prediction was out of applicability domain, which indicates greater uncertainty about its reliability. However, some possible metabolites of the chemical, based on the metabolism simulators of OASIS–TIMES, were predicted to be strong skin sensitisers.

The sensitisation potency of the chemical was predicted in a study on 229 hair dye substances using a quantitative structure activity relationship (QSAR) model, based on the LLNA. The study predicted the chemical to be a moderate to strong skin sensitiser (Sosted et al., 2004).

Repeated Dose Toxicity

Oral

Based on the available data, the chemical is not expected to cause severe effects following repeated oral exposure.

In a subchronic toxicity study, SD rats (n = 10/sex/dose) were administered the chemical by gavage doses of 0, 10, 45 or 90 mg/kg bw/day in distilled water, seven days per week, for 13 weeks. No mortalities were reported during the study. There was a slight decrease in body weight gain and food consumption in males at low and high doses. At the high dose, absolute and relative weights of the liver had slightly decreased compared with controls. Animals showed kidney discolouration and deposits and dilatation of renal pelvis and one female showed hyaline casts in the urine (cylindrical structures produced by the renal tubules). A no observed adverse effect level (NOAEL) of 45 mg/kg bw/day was determined (SCCS, 2011).

Dermal

No data are available.

Inhalation

No data are available.

Genotoxicity

Based on the results of available genotoxicity studies, the chemical is not considered to be genotoxic.

The available in vitro data showed negative results (SCCS, 2011):

- negative results in a bacterial gene mutation assay (OECD TG 471) in *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 at concentrations up to 5000 µg/plate, with and without metabolic activation;
- a mammalian gene mutation assay (OECD TG 476) with the chemical induced a marginal increase in mutant frequency at 250 µg/L without metabolic activation, but not at 375 µg/mL; the marginal increase in mutant frequency was not reproducible in further experiments, and the chemical was considered as not mutagenic in this study; and
- in a micronucleus assay (OECD TG 487) with human lymphocytes from two donors, the chemical induced a small but statistically significant increase of micronuclei at the two highest doses tested of 718.5 and 756.3 µg/mL with metabolic activation. However, the increases were within the historical control range and not reproducible in a second experiment. Thus, the chemical was considered to be negative in this study.

In an in vivo micronucleus test following OECD TG 474, the chemical was orally administered to NMRI mice (n = 5/sex/dose) at doses of 187.5, 375 or 750 mg/kg bw/day, for three consecutive days. No statistically or biologically relevant increases of micronuclei were observed in polychromatic erythrocytes collected 24 hours after the last dosing, indicating negative results (SCCS, 2011).

Carcinogenicity

Only limited data are available. There are no available reports of high quality carcinogenicity studies conducted with the chemical in animals.

Based on the available genotoxicity data, mitigating factors relating to the mechanisms of aromatic amine carcinogenicity and due to its chemical structure, this chemical is not considered to be carcinogenic.

In a skin painting study, the chemical at 0.3 % concentration in a mixture of hair dye formulations were applied to the skin (0.05 mL) of Eppley Swiss mice (n = 60/sex), three times per week, for 20 weeks. While the study concluded that none of the hair dyes tested in this study induced carcinogenicity (Jacobs et al., 1984), the SCCS stated that 'no conclusions with regard to carcinogenicity could be drawn from this study' (SCCS, 2011).

Based on QSAR predictions, the chemical, as a nitroaniline derivative, contains a structural alert for genotoxic carcinogenicity (OECD QSAR Toolbox). Nitroaniline derivatives are metabolically activated into electrophile species. This usually involves activating N-hydroxylamine metabolites and their enzymatic reaction, and eventual formation of the pro-carcinogenic nitrenium ions. The highly reactive nitrenium ions covalently bind to DNA, provided that they are sufficiently stable to not immediately undergo further reactions. The stability of the nitrenium ion is correlated with mutagenicity, for example in an Ames test with metabolic activation (Benigni & Bossa, 2011). However, the stability of the nitrenium ion depends on the type of substituents and the isomeric position of the nitro group. The combination of one nitro and one amino group attached to the same benzene ring is a structural alert for mutagenic activity, except for 2-nitroaniline derivatives (OECD QSAR Toolbox). The chemical has the nitro group attached in an *ortho*-position to the amine, which could disrupt the activation of the N-hydroxylamine metabolites. Therefore, compared with other nitroaniline derivatives, the chemical has a lower likelihood of being a carcinogen. Genotoxicity assays including Ames tests showed negative results for the chemical, and no evidence of carcinogenic activity has been reported, either in animals or humans.

Reproductive and Developmental Toxicity

Based on the results of the available teratogenicity study, the chemical up to 60 mg/kg bw/day does not show specific reproductive or developmental toxicity.

In a teratogenicity study following OECD TG 414, female SD rats (n = 24/dose) were orally dosed with the chemical at 0, 15, 30 or 60 mg/kg bw/day on gestation days (GD) 6–15. Reproductive parameters such as corpora lutea, number of implantation sites, resorption sites and number of foetuses were not affected by treatment. At the highest dose, there was a higher number of foetuses with a dilated oesophagus, but this was reported as not biologically relevant. No other effects were observed on the foetuses. No maternal toxicity was reported up to the highest dose (SCCS, 2011). However, the SCCS (2011) concluded that this study had limited value as the highest dose did not elicit maternal or foetal toxicity.

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include systemic acute oral effects and potentially local effects (skin sensitisation) at concentrations above 10 %.

Public Risk Characterisation

The chemical is used in hair dyes in Australia. The use of the chemical in hair dyes is restricted to 1 % upon application in the EU under the Cosmetics Regulation 1223/2009 Annex III (CosIng). Currently, there are no restrictions in Australia on using this chemical in hair dyes.

The SCCS (2011) has conducted a safety evaluation for repeated use of the chemical in hair dyes at 1 % concentration by a 60 kg person, under both oxidative and non-oxidative conditions. By using dermal absorption rates of 5.20 and 4.45 µg/cm2, respectively, and using a NOAEL of 45 mg/kg bw/day, the margin of safety (MOS) was calculated to be 890 for oxidative and 1125 for non-oxidative conditions. An MOS >100 is considered safe and, therefore, the SCCS Opinion (2011) recommended 'a maximum on-head concentration of 1 % in oxidative and non-oxidative hair dye formulations' as not posing a risk to the health of the consumer.

As hair dye formulations are not anticipated to contain levels of the chemical above 1 %, the MOE is not expected to be >100 for any practical hair dye concentration. Skin sensitisation is not expected at actual use concentrations.

If this chemical was included in cosmetic products containing N-nitrosating agents, the nitrosamine content should be < 50 ppb, as recommended by the SCCS (2011).

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 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 the critical acute oral and local 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.

Based on the available data, the hazard classification in the HSIS (Safe Work Australia) is considered appropriate.

NICNAS Recommendation

Current risk management measures are considered adequate to protect public and workers' health and safety, provided that all requirements are met under workplace health and safety, and poisons legislation as adopted by the relevant state or territory. No further assessment is required.

Regulatory Control

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

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Acute Toxicity	Harmful if swallowed (Xn; R22)*	Harmful if swallowed - Cat. 4 (H302)
Sensitisation	May cause sensitisation by skin contact (Xi; R43)*	May cause an allergic skin reaction - Cat. 1 (H317)

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

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

Public health

Products containing the chemical should be labelled in accordance with state and territory legislation (SUSMP, 2016).

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 and dermalexposure 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 chemicalis used. Examples of control measures that could minimise the risk include, but are not limited to:

- health monitoring for any worker who is at risk of exposure to the chemical, 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 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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A review of the physical hazards of the chemical has not been undertaken as part of this assessment.

References

Approved Criteria for Classifying Hazardous Substances [NOHSC: 1008(2004)] Third edition. Accessed at http://www.safeworkaustralia.gov.au/sites/SWA/about/Publications/Documents/258/ApprovedCriteria_Classifying_Hazardous_Substances_NOHSC1008-2004_PDF.pdf

Benigni R and Bossa C 2011. Mechanisms of Chemical Carcinogenicity and Mutagenicity: A Reviewwith Implications for Predictive Toxicology. Chem. Rev. 2011, 111, 2507-2536.

Cosmetic Ingredients and Substances database (CosIng). Accessed at http://ec.europa.eu/consumers/cosmetics/cosing/

Jacobs MM, Burnett CM, Penicnak AJ, Herrera JA, Morris WE, Shubik P, Apaja M and Granroth G 1984. Evaluation of the toxicity and carcinogenicity of hair dyes in Swiss mice. Drug Chem Toxicol 7(6), 573-586.

National Industrial Chemicals Notification and Assessment Scheme (NICNAS) 2007. List of Chemicals used as Dyes in Permanent and Semi-Permanent Hair Dyes in Australia.

Organization for Economic Cooperation and Development (OECD) Quantitative Structure-Activity Relationship (QSAR) Toolbox Software Version 3.2. September 2016.

Safe Work Australia (SWA). Hazardous Substances Information System (HSIS). Accessed July 2016 at http://hsis.safeworkaustralia.gov.au/HazardousSubstance

Scientific Committee on Consumer Safety (SCCS) 2011. Opinion on Hydroxyethyl-2-nitro-p-toluidine COLIPA n° B75. SCCS/1387/10. Adopted at the 11th plenary meeting of 21 June 2011. Available at: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_061.pdf

Scientific Committee on Consumer Safety (SCCS) 2012. Opinion on Nitrosamines and Secondary Aminesin Cosmetic Products. Adopted at its 14th plenary meeting of 27 March 2012. Accessed at http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_090.pdf

Sosted H, Basketter DA, Estrada E, Johansen JD, Patlewicz GY 2004. Ranking of hair dye substances according to predicted sensitization potency: quantitative structure-activity relationships. Contact Dermatitis 51, pp. 241-254.

The Poisons Standard (the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP)) 2016. Accessed May 2016 at https://www.comlaw.gov.au/Details/F2016L00036

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