The Director General

Maisons-Alfort, 10 December 2012

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on the application for authorisation to use cyclohexanamine,4,4'-methylenebis[N-(1-methylpropyl)- (CAS No. 154279-60-4) in the manufacture of organic coatings coming into contact with water intended for human consumption

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are made public.

On 9 May 2012 the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) received a formal request from the French Directorate General for Health (DGS) to conduct an expert appraisal in response to the application for authorisation to use cyclohexanamine,4,4'-methylenebis[N-(1-methylpropyl)- (CAS No. 154279-60-4) in the manufacture of organic coatings coming into contact with water intended for human consumption human (WIHC).

1. BACKGROUND AND PURPOSE OF THE REQUEST

The placing on the market of materials and products intended to come into contact with WIHC, and their use in facilities for the production, treatment and distribution of water, are subject to the regulatory provisions of Articles R. 1321-48 and 49 of the French Public Health Code (CSP).

The Ministerial Order of 29 May 1997, as amended, specifies the conditions to be met by materials and products used in permanent facilities for the production, treatment and distribution of WIHC. In particular, it states that organic materials can be used in contact with WIHC provided that they are made from chemical constituents authorised under the regulations on materials and products that can be placed in contact with foodstuffs, as well as those listed in Annex III of the Order.

Chapter C of the DGS's Practical Guide of March 1999 on the constitution of files relating to the health compliance of materials placed in contact with WIHC specifies which documents are required for the dossier when applying to add a new substance to one of the positive lists annexed to the Order of 29 May 1997, as amended.

The Report of December 2011 entitled "Positive Lists for Organic Materials" by the group of four European Union Member States known as the 4MS specifies the information required and describes the assessment procedure for adding a new authorised substance to the common positive list. This procedure is based on the "Note for Guidance for Food Contact Materials" issued by the European Food Safety Authority (EFSA, 2008).

2. ORGANISATION OF THE EXPERT APPRAISAL

This expert appraisal was carried out in accordance with the French Standard NF X 50-110 "Quality in Expertise – General Requirements of Competence for Expert Appraisals (May 2003)".

The collective expert appraisal was conducted by the Working Group on Assessing the safety of materials and products used in permanent facilities for the production, treatment and distribution of WIHC (MCDE WG), on the basis of a report on the applicant's technical dossier prepared by an expert from this same WG and two experts from the Expert Committee on Assessment of physico-chemical risks in food (ERCA CES) for the toxicological part of the dossier.

The analysis conducted and the conclusions reached by the MCDE WG were presented to the Working Group on Assessment of substances and processes subject to authorisation in human food (ESPA WG) and adopted by the Expert Committee (CES) on Water on 6 November 2012.

3. ANALYSIS AND CONCLUSIONS OF THE CES ON WATER

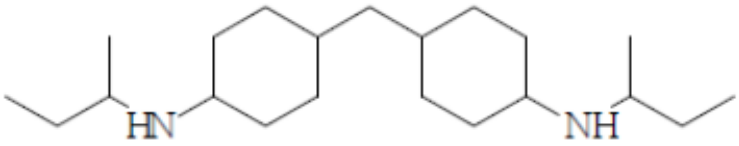
The technical dossier received from the applicant contained all the information necessary for the assessment (see Section 2.4 of the common approach recommended by the 4MS group, EFSA's "Note for Guidance" and Chapter C of the DGS Practical Guide of March 1999).

3.1. Analysis of documents received

3.1.1. Identity

Table I summarises the main data regarding the identity of the substance, which is a mixture of several isomers.

Table I: Main data regarding the identity of the substance

Name	Cyclohexanamine,4,4'-methylenebis[N-(1-methylpropyl)-
CAS number	154279-60-4
Empirical formula	$C_{21}H_{42}N_2$
Structural formula	
Molecular weight	322.57 g/mol
Purity	97.4% to 99.1%

3.1.2. Physical and chemical properties

This diamine reacts with aliphatic diisocyanates to form polyurea bonds and its physico-chemical properties are presented in Table II.

Table II: Main physico-chemical properties

Melting point	-21°C
Boiling point	355°C
Flash point	176°C
n-octanol/water partition coefficient	1.31 at 23.3°C and at pH 6.8
Solubility in water	131 µg/mL at 23°C
Density	0.9 (± 0.02) g/cm^3 at 20°C
Vapour pressure	< 0.1 kPa at 20°C
Surface tension	31.91 mN/m at 24°C

3.1.3. Intended uses

The substance is formulated into a two-component polyurea type coating designed to prevent corrosion (thickness of 1 to 2 mm) or to improve the structural properties (thickness of 3.0 to 8.5 mm) of cold WIHC distribution pipes with diameters greater than 63 mm.

The base consisting primarily of aliphatic diisocyanates and the preparation containing the diamine (referred to as the "activator") are heated separately and then mixed in equal parts during rotary spraying inside the pipes. The coating thickness is determined by the rotational speeds and the forward movement of the spray head, as well as the flow rate of the pump feeding the base and activator. Polymerisation takes one hour.

3.1.4. Authorisations for use

Cyclohexanamine,4,4'-methylenebis[N-(1-methylpropyl)- is authorised in the Netherlands with a maximum tolerable concentration at the tap (MTC_{tap}) of 9 µg/L. Under the assessment procedure for a new substance adopted by the 4MS, an authorisation with an MTC_{tap} of 9 µg/L requires the provision, in addition to genotoxicity tests, of a subchronic (90 days) oral toxicity study, which was not in the dossier submitted.

A coating containing this component is authorised for contact with WIHC in the following countries: United Kingdom, the Netherlands (for use at a maximum temperature of 35°C and in pipes with a diameter of between 100 and 610 mm), Belgium, Italy, the United States (for use at a maximum temperature of 30°C and in pipes with a diameter ≥ 4 inches (101.6 mm)), Malaysia, Taiwan and Japan.

3.1.5. Migration data

According to the NSF61 Standard (USA)
Migration tests on the coating containing cyclohexanamine,4,4'-methylenebis[N-(1-methylpropyl)- were performed according to the USA's NSF/ANSI Standard 61. No signal corresponding to cyclohexanamine,4,4'-methylenebis[N-(1-methylpropyl) was detected in the migration solutions analysed using gas or liquid chromatography coupled with mass spectrometry.

According to the XP P41-250-2 Standard, applied by the in-house laboratory
Migration tests were carried out by the applicant's in-house laboratory according to the XP P41-250-2 Standard with determination in water of cyclohexanamine,4,4'-methylenebis[N-(1-methylpropyl)- using liquid chromatography coupled with tandem mass spectrometry. After 24 h of contact, for a surface to volume (S/V) ratio of 60 cm^2/L, migration was less than 1 µg/L for the 4 samples tested. After 2 x 24 h, migration was less than 1 µg/L for one sample and 1.18 µg/L for another sample.

According to the XP P41-250-1, 2 and 3 Standards, applied by an authorised laboratory (Ministerial Order of 18 August 2009 on the conditions for authorisation of laboratories in application of Article R. 1321-52 of the French Public Health Code)*
The tests necessary to obtain an attestation of sanitary conformity (ACS) were performed with an S/V ratio of 60 cm^2/L. The results are consistent with the criteria of acceptability set by French regulations. Thus, if cyclohexanamine,4,4'-methylenebis[N-(1-methylpropyl)- were authorised in France, the coating tested could obtain an ACS.

Conclusion
Specific migration testing of cyclohexanamine,4,4'-methylenebis[N-(1-methylpropyl)-, conducted according to the USA's NSF/ANSI Standard 61 did not reveal any migration, while testing performed according to the French XP P41-250-2 Standard showed migration of less than 2.5 µg/L. Therefore, in view of the level of exposure, only the genotoxicity studies are necessary for its assessment (4MS, 2011).

However, these studies were not conducted according to the NF EN 12873-2 Standard adopted for registering a new substance on the 4MS common positive list.

3.1.6. Data on the residual content in the material in contact with the water

The actual residual content was not determined analytically. The coating formulation allows for an excess of diisocyanates in order to minimise the amount of cyclohexanamine,4,4'-methylenebis[N-(1-methylpropyl)- in the finished product.

3.1.7. Toxicological data

Genotoxicity
- Gene mutation in bacteria (Ames test) (Studies dating from 1993 and 2002)

On the basis of two studies conducted according to OECD guideline 471, and despite deviations from good laboratory practice (GLP) and experimental limitations, the results indicate a lack of mutagenic potential for the substance on this bacterial system.

- *In vitro* test for gene mutation in mammalian cells (study dating from 2011)

Despite deviations from GLP, the test for gene mutations in mouse lymphoma cells (L5178Y cells) followed most of the recommendations in OECD guideline 476 and recent

recommendations in the literature (Moore *et al.*, 2006, 2007). It indicates a lack of mutagenic potential for the substance on this cellular system.

- *In vitro* test for chromosomal aberration in mammalian cells (study dating from 2002)

Despite deviations from GLP, the *in vitro* test for chromosomal aberrations in CHL cells followed most of the recommendations in OECD guideline 473. This study clearly demonstrated the *in vitro* genotoxic potential of the substance both with and without metabolic activation on this cellular system with the induction of structural (clastogenic type action) and numerical (aneugenic type action) chromosomal aberrations. It should however be noted that the choice of cell line is questionable given its murine origin and its genetic instability that may cause false positive results (Honma and Hayashi, 2011). These results should be confirmed using genetically stable cells, such as human lymphocytes.

- Other information (study dating from 2011)

An *in vivo* genotoxicity study (micronuclei detection) conducted on mouse bone marrow cells according to OECD guideline 474 concluded that the substance is not genotoxic. However, the relevance of this finding cannot be guaranteed since the absence of erythropoiesis inhibition (no decrease in the PCE[1]/NCE[2] ratio) and determination of plasma concentrations mean it is impossible to guarantee that the target organ was actually exposed. In addition, this regulatory test is not regarded as highly sensitive (Kirkland and Speit, 2008).

General toxicity

- Combined repeated dose 28-day toxicity study and screening for toxicity to reproduction and development (study dating from 2011)

Cyclohexanamine,4,4'-methylenebis[N-(1-methylpropyl)- is a strong irritant and produced urothelial toxicity when administered to rats by oral gavage for 28 days. An adaptive response to this irritation is suggested. Females, who underwent a longer treatment duration, did not present more severe urothelial hyperplasia than males.

The parental no observed adverse effect level (NOAEL) was established at 1 mg/kg bw/day for males and 3 mg/kg bw/day for females, based on the results of the 28-day oral toxicity study.

No toxic effects on reproduction and development were observed. However, the applicant's contention that *"This study indicates that the substance does not present a risk to reproduction: the no observed adverse effect level (NOAEL) for reproduction and development has been established at more than 10 mg/kg bw/day"* should not be interpreted broadly with regard to the preliminary considerations of OECD guideline 422[3].

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[1] *PCE: polychromatic erythrocytes.*

[2] *NCE: normochromatic erythrocytes.*

[3] *The study further comprises a reproduction/developmental toxicity screening test and, therefore, can also be used to provide initial information on possible effects on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of the conceptus and parturition, either at an early stage of assessing the toxicological properties of chemicals, or on chemicals of concern. This test does not provide complete information on all aspects of reproduction and development. In particular, it offers only limited means of detecting postnatal manifestations of prenatal exposure, or effects that may be induced during postnatal exposure. Due (amongst other reasons) to the selectivity of the end points, and the short duration of the study, this method does not provide evidence for definite claims of no reproduction/developmental effects. Although, as a consequence, negative data do not indicate absolute safety with respect to reproduction and development, this information may provide some reassurance if actual exposures were clearly less than the dose related to the No Observed Adverse Effect Level (NOAEL).*

- Other information

The 50% lethal dose (LD_{50}) after dermal exposure has been set at 1600 to 2000 mg/kg on the basis of an acute toxicity study in rats conducted in 2005.

The 50% lethal dose (LD_{50}) after oral exposure has been set at 227 mg/kg on the basis of an acute oral toxicity study in rats conducted in 2001.

Cyclohexanamine,4,4'-methylenebis[N-(1-methylpropyl)- is corrosive to rabbit skin according to the results of a test conducted in 1993 and is classified in the regulations as a skin sensitiser based on a Local Lymph Node Assay (LLNA) dating from 2005 which was not included in the dossier but is mentioned in the chemical safety report.

Conclusion

The studies followed most of the recommendations in the corresponding OECD guidelines. However, none of the studies provided a control for the concentrations in the treatment formulations with the solvents/excipients used, which, as well as being a deviation from the GLP, means that the stability of the product under the treatment conditions cannot be guaranteed.

In view of all these factors, it is not possible to conclude as to the lack of genotoxic potential of cyclohexanamine,4,4'-methylenebis[N-(1-methylpropyl)-. Consequently, without the provision of precise additional data, the genotoxic risk to humans cannot at this stage be ruled out.

3.2. Conclusions

In view of the dossier submitted by the applicant, the CES on Water:

1) is issuing a stay of proceedings on the application for authorisation to use cyclohexanamine,4,4'-methylenebis[N-(1-methylpropyl)- (CAS No. 154279-60-4) in the manufacture of organic coatings coming into contact with water intended for human consumption;

2) is asking for the *in vitro* chromosomal aberration test to be repeated in a study performed according to good laboratory practice (GLP) using genetically stable cells of human origin (e.g. human lymphocytes) (OECD 473).

   If the results obtained in this new study on this new cell type are negative, the assumption of a false positive result obtained in the p53-deficient, genetically unstable murine CHL line may be advanced.

   However, in the event of positive or equivocal results on the frequency of structural and numerical aberrations, further investigation *in vivo* will be necessary. Given the possible genotoxic mechanisms of action, the test(s) performed should enable clastogenic and/or aneugenic type genotoxic events to be taken into account on one or more target organs. The following tests may be performed:
   - the alkaline version of a comet assay able to show various types of DNA damage (single and double-stranded breaks, alkali-labile sites, sites of incomplete DNA repair, etc.). As effects have been demonstrated both in the absence and presence of metabolic activation, the study should focus on a systemic organ capable of metabolisation (e.g. the liver), but also on a local organ of interest based on the expected oral exposure in humans, for

example an organ of the gastrointestinal tract (stomach and/or colon and/or duodenum). The test should be carried out taking into account the recent recommendations in the literature defining the optimal conditions for its implementation (Tice *et al.*, 2000; Hartmann *et al.*, 2003, 2004; Burlinson, 2007).

- as the comet assay is not the most suitable for detecting aneugenic compounds, it should be coupled with the micronucleus assay, as has been proposed in several publications (Pfuhler *et al.*, 2007; Vasquez, 2010), on an organ of interest (OECD 474). Given the type of exposure expected in humans, conducting the assay on the colon seems relevant.

Negative results for both assays would confirm that the test compound is not genotoxic *in vivo*.

For all of these tests, the concentrations in the treatment formulations should be verified.

If migration tests, conducted according to the NF EN 12873-2 Standard (4MS, December 2011), showed migration in water greater than 2.5 µg/L, additional toxicological data would be required after 90 days of exposure.

4. AGENCY'S CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety adopts the conclusions and recommendations of the CES on Water.

The Director General

Marc Mortureux

KEY WORDS

Water intended for human consumption, water contact materials, organic materials, positive lists, authorisation of a substance.

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4.2. Standards

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NF EN 12873-2: Influence of materials on water intended for human consumption – Influence due to migration – Part 2: Test method for non-metallic and non-cementitious site-applied materials.

NSF/ANSI Standard 61: NSF International Standard / American National Standard / Drinking Water System Components – Health Effects.

XP P 41-250-2: Effects of materials on the quality of water intended for human consumption – Organic materials – Part 2: Measurement method for mineral and organic micropollutants.

4.3. Legislation and Regulations

Ministerial Order of 29 May 1997 on materials and products used in permanent facilities for the production, treatment and distribution of WIHC, as amended by the Orders of 24 June 1998, 13 January 2000, 22 August 2002 and 16 September 2004 (published in the Official Journals of 1 June 1997, 25 August 1998, 21 January 2000, 3 September 2002 and 23 October 2004).

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