The Director General

Maisons-Alfort, 28 May 2014

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

on the application for authorisation to use isododecane in the manufacture of organic materials coming into contact with drinking water

ANSES undertakes independent and pluralistic scientific expertise.
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.
This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 28 May 2014 shall prevail.

On 5 February 2014, 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 isododecane in the manufacture of organic materials coming into contact with drinking water (DW).

1. BACKGROUND AND PURPOSE OF THE REQUEST

The placing on the market of materials and products intended to come into contact with DW, 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 DW. In particular, it states that organic materials can be used in contact with DW 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 DW 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 (4MS, 2011). This procedure is based on the "Note for Guidance for Food Contact Materials" issued by the European Food Safety Authority (EFSA, 2008).

On 3 January 2013, the Agency issued a stay of proceedings on the application for authorisation to use isododecane (CAS nos: 13475-82-6 / 93685-81-5) in the manufacture of organic materials coming into contact with drinking water. Indeed, the results of the *in vitro* chromosomal aberration test in Chinese hamster V79 cells (RCC Study 524402 of 03/06/1996) had shown there were chromatid-type exchanges considered biologically significant. Since this test was undertaken in a karyotype-unstable murine cell line (Chinese hamster lung V79 cells), it was concluded that this result needed to be confirmed by a new study using genome-stable human cells. The Agency asked for the *in vitro* chromosomal aberration test (OECD 473) to be repeated in a study complying with rules of good laboratory practice (GLP), using genetically stable cells of human origin such as human lymphocytes (Opinion No 2012-SA-0143).

The applicant proposed to undertake the *in vitro* micronucleus test in human lymphocytes according to OECD Guideline 487 instead of the requested metaphase analysis test. Since the *in vitro* micronucleus test (OECD 487, 2010) is capable of highlighting both clastogenic and aneugenic phenomena, this proposal was accepted. Moreover, the '4MS' group[1] agreed that the three genotoxicity tests required in the common approach could be replaced by two genotoxicity tests: the Ames test (OECD 471) and the *in vitro* micronucleus test (OECD 487) as recommended by EFSA (EFSA, 2011) and COM[2] (COM, 2011). In addition, the Agency recommended undertaking the *in vitro* micronucleus test in human cells as advocated in the literature (Honma, 2010; COM, 2011; Fowler, 2009; Fowler *et al.*, 2009), even though the OECD Guideline 487 mentions the possibility of using rodent cells such as CHO, V79, CHL and L5178Y cells.

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 DW (PDWs WG), on the basis of a report on the applicant's technical dossier prepared by an expert from the Expert Committee on Assessment of chemical risks in food (ERCA CES).

The analysis conducted and the conclusions reached by the PDWs 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 May 2014.

[1] SG-OM 13-15: Minutes of the meeting of 31 January 2013 of the 4MS Sub-Group Organic Materials.

[2] COM: Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment.

ANSES analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals. The experts' declarations of interests are made public on ANSES's website (www.anses.fr).

3. ANALYSIS AND CONCLUSIONS OF THE CES ON WATER

3.1. Analysis of documents received

Genotoxicity

- *In vitro* micronucleus test in human lymphocytes (Study 8287-229 of 09/01/2014 undertaken by the Covance laboratory (France), purity of isododecane: 99.87%)

The *in vitro* micronucleus test in human lymphocytes was undertaken in accordance with OECD Guideline 487.

The tests were undertaken with and without metabolic activation:
- With metabolic activation, the cells were treated for 3 hours followed by 21 hours or 45 hours of recovery, without treatment;
- Without metabolic activation, the cells were treated for a short time of 3 hours followed by 21 hours or 45 hours of recovery, or for a long time of 20 hours followed by 24 hours of recovery.

One thousand cells per culture (i.e. 2000/concentration) were examined in order to determine the frequency of micronucleated cells.

Isododecane tested at its limit of solubility (85 µg/mL) did not induce a statistically significant increase in the frequency of micronucleated cells either with or without metabolic activation and was therefore considered non-genotoxic in these operational conditions.

The study followed most of the recommendations in the OECD guidelines. However, no control was provided for the concentrations in the treatment solutions in ethanol which, as well as being a deviation from GLP, means that the product's stability under treatment conditions cannot be guaranteed. However, this limitation is not likely to call into question the findings of the study.

- Conclusion

In addition to the cell type used, there were significant methodological differences between the *in vitro* chromosomal aberration test in V79 cells (RCC Study 524402 of 03/06/1996, see Opinion 2012-SA-0143) and this study, particularly regarding the concentrations actually studied in terms of genotoxicity. Indeed, the concentrations studied in the *in vitro* chromosomal aberration test in V79 cells were much higher and chromatid-type exchanges were observed at the concentrations of 500 and 3000 µg/mL with metabolic activation. There was no information regarding the appearance of precipitate in the previous study. Yet in the additional study (Covance Study 8287-229), solubility was precisely the limiting factor, with a maximum concentration of 85 µg/mL tested with metabolic activation.

However, since this study was undertaken in accordance with OECD Guideline 487 (2010) and used primary human cells, its results override those obtained previously in the p53-deficient,

genetically unstable murine V79 line. The assumption of a false positive result for this latest study could be advanced.

In light of all of the *in vitro* mutagenesis and genotoxicity studies, isododecane has no mutagenic effects on bacteria (Ames test, CCR Study 161908 of 17/01/1990, see Opinion 2012-SA-0143) or on mammal cells (HGPRT test, RCC Study 524401 of 27/03/1996, see Opinion 2012-SA-0143), nor any genotoxic effects on human lymphocytes (*in vitro* micronucleus test, Covance Study 8287-229 of 09/01/2014). These three *in vitro* tests appear sufficient to conclude that isododecane is not genotoxic or mutagenic.

3.2. Conclusions

In view of the dossier submitted by the applicant, the CES on Water is issuing a favourable opinion on the application for authorisation to use isododecane (CAS nos: 13475-82-6 / 93685-81-5) in the manufacture of organic materials coming into contact with drinking water with a maximum allowable concentration at the consumer's tap (MTC_{tap}) of 2.5 µg/L.

The CES on Water notes the significance of using isododecane with the same impurity profile or greater purity than that used for the toxicity tests in the manufacture of organic materials coming into contact with DW.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

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

Marc Mortureux

KEYWORDS
Drinking water, water contact materials, organic materials, positive lists, authorisation of a substance.

REFERENCES

4.1. Publications

4MS (December 2011). Positive Lists for Organic Materials – 4MS Common Approach – Part A: Compilation and management of a suite of Positive Lists (PLs) for organic materials – Part B: Assessment of products for compliance with Positive List requirements (Conversion Factors – CFs).
www.umweltbundesamt.de/sites/default/files/medien/419/dokumente/4ms_positive_list_0.pdf

COM (2011). Guidance on a strategy for genotoxicity testing of chemical substances.
www.iacom.org.uk/guidstate/documents/COMGuidanceFINAL.pdf

DGS (March 1999). Guide pratique pour la constitution des dossiers relatifs à la conformité sanitaire des matériaux placés en contact avec les eaux d'alimentation (Practical Guide on the constitution of dossiers relating to the health compliance of materials placed in contact with drinking water).
www.sante.gouv.fr/fichiers/bo/1999/99-25/a0251660.htm

EFSA (30 July 2008). Note for guidance for petitioners presenting an application for the safety assessment of a substance to be used in food contact materials prior to its authorisation.
www.efsa.europa.eu/fr/efsajournal/doc/21r.pdf

EFSA (2011). Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment. *EFSA Journal 2011*, 9(9), 2379.
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Fowler P., Williams K., Jeffrey L., Carmichael P., Ardema M., Diembeck W., Fautz R., Harvey J., Hewitt N., Latil A. *et al.* (2009). Reduction of misleading ("false") positive results in mammalian cell genotoxicity assays I. Choice of cell type. *Environmental Molecular Mutagenesis*, 50, 572.

Honma M., Hayashi M. (2011). Comparison of *in vitro* micronucleus and gene mutation assay results for p53-competent versus p53-deficient human lymphoblastoid cells. *Environ Mol Mutagen.*, 52(5), 373-84.

4.2. Standards

OECD 487 (22 July 2010). OECD Guideline for the Testing of Chemicals – *In Vitro* Mammalian Cell Micronucleus Test.

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 water intended for human consumption, 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).