The Director General

Maisons-Alfort, 3 January 2013

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 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 8 June 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 isododecane in the manufacture of organic materials coming into contact with water intended for human consumption (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

The expert appraisal was carried out in accordance with 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 an expert 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 4 December 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

Isododecane corresponds to a group of linear and/or branched saturated hydrocarbons composed exclusively of hydrogen and carbon (general formula $C_{12}H_{26}$). Theoretically, there are 355 isododecane isomers.

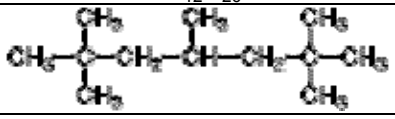
When obtained synthetically, through an oligomerisation reaction at 120°C on an isobutylene acid resin followed by catalytic hydrogenation, and not by the fractional distillation of petroleum, it is highly branched which gives it greater chemical inertness.

Table I summarises the main data regarding the identity of the substance when it is obtained by synthesis. The main isomers are:

- 83% 2,2’,4,6,6’-pentamethylheptane (CAS No: 13475-82-6),
- 7% 2,2’,4,5,6-pentamethylheptane (CAS No: 62199-64-8),
- 3% 2,2’,4,4’,6-pentamethylheptane (CAS No: 62199-62-6),
- 0.6% 2,2’,3,6,6’-pentamethylheptane (CAS No: 62198-88-3).

In the safety data sheet (SDS) attached to the dossier and in the chemical safety report submitted to the European Chemicals Agency (ECHA) under the REACH Regulation, the substance is identified as "Hydrocarbons, C4, 1,3-butadiene-free, polymd., triisobutylene fraction, hydrogenated" (CAS No: 93685-81-5). It is described as "UVCB": substances of unknown or variable composition, complex reaction products or biological materials.

Table I: Main data regarding the identity of the substance

Name	Isododecane or hydrocarbons, C4, 1,3-butadiene-free, polymd., triisobutylene fraction, hydrogenated
CAS number	93685-81-5
Empirical formula	$C_{12}H_{26}$
Semi-structural formula for 2,2',4,6,6'-pentamethylheptane	
Molecular weight	170 g/mol
Purity obtained by oligomerisation at 120°C on isobutylene resin acid leading to the formation of triisobutylene (TIB) followed by catalytic hydrogenation	99.5% C_{12} isomers 83% 2,2',4,6,6'-pentamethylheptane (CAS No: 13475-82-6) Contains no sulphur (<1 ppm), carbonyl (<5 ppm), aromatic molecules (<1 ppm) or naphthenic molecules

3.1.2. Physical and chemical properties

Isododecane's physico-chemical properties are presented in Table II.

Table II: Main physico-chemical properties

Melting point	- 81°C
Boiling point	176-192°C
Flash point	45°C
n-octanol/water partition coefficient (Log $K_{o/w}$)	6.32
Density	0.75 (20°C)
Vapour density	5.9 (Air=1)
Vapour pressure	1.1 hPa (20°C) 3.4 hPa (50°C)
Surface tension	22.6 mN/m (20°C)
Viscosity	1.30 mPa.s (20°C)

3.1.3. Intended uses

Isododecane is used as a phlegmatiser for organic peroxides used in radical polymerisation for the production of many polymers, such as polyvinyl chloride (PVC), low-density polyethylene (LDPE), ethylene-vinyl acetate copolymers, ethylene-acrylate copolymers, ethylene-acrylate-maleic anhydride terpolymers, and certain acrylic polymers used as impact additives for PVC, rubber and elastomers. Isododecane is also used as a transfer or rinsing agent for organic peroxides in reactor polymerisation.

Isododecane is typically used at less than 0.1% by weight in materials.

3.1.4.Authorisations for use

Isododecane is authorised in Germany with a maximum tolerable concentration at the tap (MTC_{tap}) of 250 µg/L (http://kse.zadi.de/kse/faces/DBEmpfehlung.jsp):

- BfR[1] II "*Plasticizer-free Polyvinyl Chloride, Plasticizer-free Copolymers of Vinyl Chloride and Mixtures of these Polymers with other Copolymers and Chlorinated Polyolefins Containing Mainly Vinyl Chloride in the Total Mixture (As of 01.01.2012)".* Section 2a: "*For the catalysts mentioned below isododecane may be added as desensitising agent. The transfer from the final product may not exceed 5 mg isododecane/kg foodstuff".*
- BfR III "*Polyethylene (As of 01.03.2011)*". Section 2b: "*tert-Amyl perneodecanoate with isododecane as desensitizing agent. The maximum amount of tert-amyl perneodecanoate is 560 mg/kg of polymer and of isododecane 190 mg/kg of polymer*".
- BfR V "*Polystyrene Produced Exclusively from the Polymerisation of Styrene (As of 01.01.2010)".* Section 2a: "*O,O-tert-Butyl-O-isopropyl-monoperoxycarbonate, max. 0.05%, 50% isododecane can be added as a desensitizing agent*".
- BfR VI "*Styrene Copolymers and Graft Polymers, and Mixtures of Polystyrene with other Polymers (As of 01.01.2010)*". Section 2a: "*O,O-tert-Butyl-O-isopropyl-monoperoxycarbonate, max. 0.05%; 50% isododecane may be added as a desensitizing agent*".
- BfR XXXV "*Copolymers of Ethylene, Propylene, Butylene, Vinyl Esters and Unsaturated Aliphatic Acids, and their Salts and Esters (As of 01.01.2010)".* Section 2a: "*tert-Amylperpivalate, max. 0.014%, in mixture with isododecane, max. 0.005% and tert-Amylperneodecanoate, max. 0.02%, in mixture with isododecane, max. 0.007%*".
- Letter from the BfR "*following listed catalysts can be used in mixtures with Isododecane as a desensitizing agent, as long as the specific migration limit of 5 mg Isododecane/kg food is not exceeded*".

Hydrocarbon solvents are authorised for the manufacture of materials in contact with water:

- In the Netherlands: C_{10}-C_{14} hydrocarbons with an aromatic content ≤ 1% (*Dutch warenwet Hoofdstuk I, 22-11-2006, p. 88, "Koolwaterstoffen, C10-C14, aromaatgehalte ≤ 1%*)
- in the United States: the substance (*Isoparaffinic petroleum hydrocarbons, synthetic)* complies with Section 178.3530 of Regulation FDA 21 CFR (www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm).

The DGS ruled as inadmissible the reference to the Council of Europe Resolution AP(92)2 on aids to polymerisation to authorise isododecane as a phlegmatiser[2].

3.1.5. Migration data

Migration tests were carried out by an authorised laboratory (Ministerial Order of 18 August 2009 on the conditions for the authorisation of laboratories under Article R*. 1321-52 of the French Public Health Code) in accordance with the XP P41-250-2 Standard with determination of isododecane in water by gas chromatography coupled to mass

[1] *Bundesinstitut für Risikobewertung* (German Federal Institute for Risk Assessment)

[2] *Correspondence of 17 July 2012 for the attention of authorised laboratories. Organic materials coming into contact with water intended for human consumption - Exemption from applicable regulations for the use of isododecane in plastic material formulations coming into contact with water.*

spectrometry (GC-MS). After 24 h of contact, for a surface area to volume ratio (S/V) of 240 cm^2/L, migration was below the method's limit of detection (LOD) (0.2 µg/L) for the three PVC samples tested. Thus, in view of the level of exposure (migration less than 2.5 µg/L), only genotoxicity studies are required for its evaluation.

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 water

The residual level of isododecane in two PVC samples was determined by GC-MS after extraction with methanol by heating for 15 min at 130°C on ground materials. Concentrations were below the method's LOD (400 µg/L).

3.1.7. Toxicological data

Genotoxicity

- Gene mutation in bacteria (Ames test) (studies dating from 1990 that provide no information on the purity of isododecane)

The study conducted according to OECD Guideline 471 suggested a lack of mutagenic potential for isododecane on the bacterial system used for the Ames test. However, there were significant limitations, in particular the failure to use a specific strain of *Salmonella* Typhimurium (e.g. strain TA102) to highlight certain genetic events. Thus, these results should be used with caution.

- *In vitro* test for gene mutation in mammalian cells (study dating from 1996 in which the purity of isododecane is at least 98% C_{12} isoparaffin with a maximum of 2% C_8-C_{16} isoparaffin)

The hypoxanthine-guanine phosphoribosyltransferase (HGPRT) locus gene mutation assay on Chinese hamster lung cells (V79 cells), according to OECD Guideline 476, in the presence and absence of metabolic activation, suggests a lack of mutagenic potential for isododecane on this cellular system. However, in the absence of a test using long treatment times without metabolic activation, these results should be used with caution.

- *In vitro* test for chromosomal aberration in mammalian cells (study dating from 1996 in which the purity of isododecane is at least 98% C_{12} isoparaffin with a maximum of 2% C_8-C_{16} isoparaffin)

Despite deviations from good laboratory practice (GLP), the *in vitro* test for chromosomal aberrations in Chinese hamster lung cells followed most of the recommendations in OECD Guideline 473. No statistically significant and reproducible increase was noted in the number of chromatid-type and chromosomal structural aberrations. However, chromatid exchange type aberrations were detected in the second test in the presence of metabolic activation. These clastogenic effects, which correspond to a double genetic event (breakage and rearrangement), should be considered biologically significant. Indeed, the lack of effect in the first test is not sufficient evidence to conclude that isododecane does not induce structural chromosomal aberrations in this cell system, because the type of chromosomal aberrations observed is relatively rare, including with reference clastogenic substances. The choice of cell line is questionable given its murine origin and genetic instability, which may cause false positive results (Honma and Hayashi, 2011). These results should be confirmed using genetically stable cells, such as human lymphocytes.

General toxicity

- 90-day subchronic oral toxicity study (study dating from 1983 with isododecane purity of 84.9% 2,2',4,6,6'-pentamethylheptane)

This study makes no reference to compliance with any OECD guideline and it seems that it was conducted without due regard to GLP recommendations.

Isododecane is quite well tolerated by Wistar rats exposed by oral gavage for 90 days, with no treatment-related mortality up to a maximum dose of 3000 mg/kg/day (administered doses: 330, 1000 and 3000 mg/kg/d). The decrease in body mass was more pronounced in male rats (from the first week at 3000 mg/kg/d and from the fourth week at 1000 mg/kg/d) than in female rats (from the 11th week at 3000 mg/kg/d).

Histopathological changes corroborated by biochemical blood and urine analyses indicate that the target organs are the liver, kidneys and adrenal glands. Nephrotoxic effects observed exclusively in male rats may be partly related to the nature of isododecane and have already been observed with other substances such as d-limonene (NTP, 1990; Flamm and Lehman-McKeeman, 1991; Lehman-McKeeman and Caudill, 1994). This mechanism, involving the α2µ-globulin protein, is very specific to male rats. Haematotoxicity at doses greater than 1000 mg/kg/d has also been observed in male rats only.

The no observed adverse effects level (NOAEL) has been established at 330 mg/kg bw/d for males and females, based on the results of this 90-day oral toxicity study.

Conclusion

Studies did not always follow the corresponding OECD guidelines. Irrespective of the study, no control was provided for the concentrations in the treatment solutions with the solvents/excipients used, which, as well as being a deviation from GLP, means that the product's stability under 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 isododecane. 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 isododecane (CAS nos: 13475-82-6 / 93685-81-5) in the manufacture of organic materials 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). A control should be provided for the concentrations in the treatment solutions used during this study.

   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 V79 line may be advanced.

3) reiterates that the level of isododecane migration should be verified by testing according to the NF EN 12873-2 Standard (4MS, December 2011), to enable it to be registered on the 4MS common positive list.

4. AGENCY 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.

BIBLIOGRAPHY

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/wasser-e/themen/downloads/trinkwasser/4ms_positive_list.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 for the constitution of files relating to the health conformity of materials that come into contact with drinking water).
www.sante.gouv.fr/rese/edch/reg/ti-a020.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/en/scdocs/doc/21r.pdf.

Flamm W.G., Lehman-McKeeman L.D. (1991). The human relevance of the renal tumor-inducing potential of d-limonene in male rats: implications for risk assessment. *Regul. Toxicol. Pharmacol.*, 13, 1:70-86.

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.

Lehman-McKeeeman L., Caudill D. (1994). d-Limonene Induced Hyaline Droplet Nephropathy in {alpha} 2u-Globulin Transgenic Mice. *Fundam. Appl. Toxicol,* 23, 4:562-568.

NTP (1990). Toxicology and Carcinogenesis Studies of d-Limonene (CAS No. 5989-27-5) in F344/N Rats and B6C3F1 Mice (Gavage Studies).
www.ncbi.nlm.nih.gov/pubmed/12704437.

4.2. Standards

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.

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).

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.

Council of Europe Resolution AP (92)2 on control of aids to polymerisation (technological coadjuvants) for plastics materials and articles intended to come into contact with foodstuffs (adopted by the Committee of Ministers on 19 October 1992 at the 482nd meeting of the Ministers' Deputies).