SCIENTIFIC OPINION

Scientific Opinion on the safety evaluation of the substance, dioctadecyl disulphide, CAS No 2500-88-1, for use in food contact materials

EFSA Panel on food contact materials, enzymes, flavourings and processing aids (CEF)2, 3

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This scientific opinion of EFSA deals with the safety assessment of the additive dioctadecyl disulphide, CAS No 2500-88-1, REF. No 49840; FCM substance No 449 for which the CEF Panel concluded that a limited daily intake of the substance up to 0.05 mg/person is unlikely to be a safety concern.

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KEY WORDS

Dioctadecyl disulphide; Distearyl disulphide; CAS number 2500-88-1; Ref. No 49840; FCM substance No 449; Food contact materials; Safety assessment; Evaluation.

1 On request from the DG SANCO, Brussels, Question No EFSA-Q-2011-00079, adopted on 29 September 2011.
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3 Acknowledgement: The Panel wishes to thank the members of the Working Group on Food Contact Materials for the preparation of this opinion: Mona-Lise Binderup, Laurence Castle, Riccardo Crebelli, Roland Franz, Nathalie Gontard, Eugenia Lampi, Jean-Claude Lhuguenot, Maria Rosaria Milana, Karla Pfaff, Kettil Svensson and Detlef Wölfle for the support provided to this EFSA scientific output.

SUMMARY

The EFSA was requested by the European Commission to assess the safety in use of the substance dioctadecyl disulphide and if the established specific migration limit (SML) of 3 mg/kg food is still valid on the basis of new information provided by industry.

The substance is used as an additive in plastics intended to come in contact with food and it was authorised in 2001 following an opinion by the Scientific Committee on Food (SCF) published in 1995. In that opinion a TDI of 0.05 mg/kg bw was allocated to the substance based on the results of a 90-day oral rat study of 1969 submitted by the industry. The Commission allocated an SML of 3 mg/kg food on the assumption that a person, weighing 60 kg, may consume daily up to 1 kg of food in contact with a food contact material containing the substance.

In 2010 the industry submitted new toxicological studies to the Commission with the question to be examined if the substance is accumulated in man.

The Commission asked the EFSA to review the information provided and advise on whether it raises issues regarding the safe use of the substance and to assess if the SML of 3 mg/kg food is still appropriate.

Data from two toxicokinetic studies in male rats suggest that dioctadecyl disulphide is poorly absorbed from the gastrointestinal tract.

The CEF Panel considers that there is no concern regarding the genotoxicity of dioctadecyl disulphide based on negative findings in five in vitro genotoxicity assays and one in vivo micronucleus assay.

Two 90-day toxicity studies in which dioctadecyl disulphide was administered orally to rats and beagle dogs could not be used for the derivation of a NOAEL/LOAEL due to the limited data reporting (rat study) and inadequate study design (dog study).

In two chronic studies deposits of dioctadecyl disulphide were observed in several organs (e.g. liver, adrenals, lymph nodes) in rats and dogs, together with treatment-related evidence of toxicity. An inverse dose:response relationship was however seen for both deposition of test material and organ changes evidencing toxicity. The Panel considered that due to absence of a positive dose-relationship for the observed effects and the presence of possible treatment-related effects at the lowest dose tested, the results of the study could not be used for the purposes of risk assessment of dioctadecyl disulphide.

In a reproduction/developmental toxicity study the NOAEL for reproductive toxicity was at the highest administered dose (approximately 1200 mg/kg bw/day).

The CEF Panel concluded that dioctadecyl disulphide is very poorly absorbed from the gastrointestinal tract, but is taken up to some extent as evidenced by the finding of insoluble test material in various organs in chronic studies in two species. The substance therefore has a potential for accumulation, and to result in toxicity. Due to uncertainties related to the dose-response relationship and the presence of possible treatment-related effects at the lowest dose tested in two species, the Panel could not derive a NOAEL for dioctadecyl disulphide from the available in vivo studies.

Therefore the CEF Panel does not concur with the TDI of 0.05 mg/kg/bw for dioctadecyl disulphide allocated by the SCF in 1995 and with the SML of 3 mg/kg food based on this TDI. However, considering the absence of genotoxicity, a limited daily intake of the substance up to 0.05 mg/person is unlikely to be a safety concern.
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BACKGROUND AS PROVIDED BY THE COMMISSION

Dioctadecyl disulphide (PM ref No 49840) was authorised in 2001 to be used as an additive for the manufacture of plastic food contact materials and is currently listed in Annex III B of Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with foodstuffs, with a Specific Migration Limit (SML) of 3 mg/kg food.

The authorisation for the use of this substance was granted on the basis of a positive opinion given by the Scientific Committee on Food (SCF) in 1995. This scientific opinion was based on a report made by the company Hoechst in 1969. We are not in possession of the mentioned report. The Tolerable Daily Intake was established at 0.05 mg/kg bw after a 90-day oral rat study.

The speciality chemicals business of Hoechst was bought by Clariant Produkte (Deutschland) GmbH in 1997. On 17 February 2010 the company Clariant Produkte (Deutschland) GmbH informed the Commission about their doubts regarding the possible bioaccumulation of the substance due to its high lipophilicity and limited water solubility. Following our requests of 29 July 2010 and 11 August 2010, Clariant Produkte (Deutschland) GmbH has provided the Commission on 29 November 2010 with an expert review and the available reports.

This information has to be considered in the framework of Article 11(5) of Regulation (EC) No 1935/2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC, which foresees that: "The applicant or any business operator using the authorised substance or materials or articles containing the authorised substance shall immediately inform the Commission of any new scientific or technical information, which might affect the safety assessment of the authorised substance in relation to human health. If necessary, the Authority shall then review the assessment."

TERMS OF REFERENCE

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, the European Commission would like to ask the Authority to review the information provided and advise on whether it raises issues regarding the safe use of the substance and to assess if the SML of 3 mg/kg food is still appropriate.
ASSESSMENT

1. Introduction

According to the industry dioctadecyl disulphide is used up to 0.5% w/w in polypropylene or polypropylene copolymers as an antioxidant in combination with phenolic antioxidants to improve the ageing resistance of plastics.

The substance was evaluated in the past by the SCF which set a TDI of 0.05 mg/kg bw based on the results of a 90-day oral rat study of 1969 submitted by the industry (EC, 1995).

Following this opinion, the Commission allocated an SML of 3 mg/kg food on the assumption that a person, weighing 60 kg, may consume daily up to 1 kg of food in contact with a food contact material containing the substance (EC, 2001).

In 2010 the industry submitted new toxicological studies to the Commission with the question to be examined if the substance is accumulated in man.

2. Legislation

Dioctadecyl disulphide is authorised as an additive in plastics for contact with food. It is listed with an SML of 3 mg/kg food in Annex I of the Commission Regulation (EU) No 10/2011 relating to plastic materials intended to come into contact with foodstuffs (EU, 2011).

3. Chemistry

Dioctadecyl disulphide, synonym distearyl disulphide, CAS No 2500-88-1, has a molecular weight of 571.1 g/mol. The hydrocarbon chains in the molecule are C18 in a percentage of 95-99%. Other hydrocarbon chains present are C14, C16 and C20.

Chemical formula: C36H74S2

Chemical structure:

The substance is solid at room temperature with a melting point in the range of 52-58 °C. The substance is soluble in organic solvents such as pentane, diethyl ether, chloroform, benzol. It is sparingly soluble in distilled water, 2.8 mg/100 ml, while it has very limited solubility in small aliphatic alcohols such as: methanol, ethanol and butanol.

The substance is thermally stable and no degradation or reaction products are expected during processing of the polymer.

4. Toxicity

4.1. Genotoxicity

Dioctadecyl disulphide was tested for genotoxicity in five in vitro assays and one in vivo test. The Panel noted that the substance had limited solubility in the solvents and media used in these tests. However the maximum concentration used in the tests fulfilled the requirements of the OECD guidelines. Therefore the studies were considered valid. In the bacterial reversion assays using the Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA 1538 and the Escherichia coli stain WP2uvrA, the test substance was not mutagenic. In mammalian cell gene mutation tests (L5178Y/TK+- mouse lymphoma assay and an UDS assay using the human lung cell line A549 cells), no dose related increases of the mutant frequency or induction of unscheduled DNA synthesis were observed. No indication for chromatid damage was observed in a sister chromatid exchange
Dioctadecyl disulphide

assay (CHO cells). An erythrocyte micronucleus test with male and female mice did not show any indication for genotoxicity in vivo. In conclusion, the CEF Panel considered that there is no concern regarding the genotoxicity of dioctadecyl disulphide.

4.2. 90-day oral studies
In two 90-day toxicity studies dioctadecyl disulphide was administered orally to rats (at 0.1% and 1% in diet; 10 males and 10 females per group) and beagle dogs (12000 mg/kg in the diet; 2 males and 2 females were treated; no concomitant controls). Female rats in the 0.1% group had lower body weights compared to controls. In treated female rats increased weights of liver, spleen and ovaries were observed without a clear dose-dependency. In the dog study no treatment-related effects were observed for body weight gain, clinical signs, haematology, clinical chemistry, urinalysis and organ weights. The liver function test (retention of bromosulphthalein) deviated for one female possibly indicating a transient liver effect. Due to the limited data reporting (rat study) and inadequate study design (dog study), the results could not be used for the derivation of a NOAEL/LOAEL.

4.3. Chronic oral studies
In a chronic study (involving pretreatment of parent animals and extended treatment time of the offspring, i.e. 130 weeks) rats were fed with 0.6, 1.2 and 2.4 % of dioctadecyl disulphide in the diet (corresponding roughly to 300, 600 and 1200 mg/kg bw/day). From treatment week 112 onwards, mortality in high dose males was twice as high as in controls. From treatment week 20 onwards, significantly decreased body weights of treated animals from all dose groups were observed compared to control values, with a more pronounced difference in animals of the low and mid dose groups. No difference in food consumption was observed between treated and control animals. Decreases in red blood cell parameters were reported in all groups of treated females, and increases in neutrophil counts, partially accompanied by increased white blood cell counts were observed in both males and females in all treatment groups. Urine volumes increased in the high dose groups of males (at weeks 26, 52 and 102: ca. 40-50%) and females (at week 102: 35%). Increased organ weights (liver, spleen and gonads) were observed along with pathological (enlargement and discoloration in spleen and mesenteric lymph nodes) and histopathological findings (accumulation of crystalline material, in a follow up study confirmed to be the test substance, in the liver, spleen, ovaries, bone marrow (sternum), mesenteric lymph nodes and adrenals, usually accompanied by multinucleated giant cells) in all treatment groups. The effects (including the accumulation of crystalline material in tissues) were more pronounced in the low and mid dose groups than in the high-dose group (e.g. the relative liver weights at the low, mid and high doses were 4.06, 3.60, 3.43 /100 g bw vs. 3.13 g/100 g bw in controls for males and 4.97, 4.31, 3.83 vs. 3.54 g/100 g bw for females). Despite the lack of a positive dose-response relationship, the CEF Panel concluded that the changes described might be treatment-related. Neoplastic changes (hemangio-endotheliomas in the mesenteric lymph nodes: 8 at the low dose vs. 1 in controls and the other treatment groups for males and 3, 5 and 1 at the low, mid and high dose, respectively, vs. 0 in controls for females) were found in animals of the low and mid dose groups. However, the neoplastic lesions developed in separate areas within the affected lymph nodes and therefore might not be causally linked to the accumulation of the micro-crystalline material.

The CEF Panel considered that this chronic study in rats provided evidence of accumulation of the test substance in rat tissues. The Panel considered however that due to absence of a positive dose-relationship for the observed effects and the presence of possible treatment-related effects at the lowest dose tested, the results of the study could not be used for the purposes of risk assessment.

Additionally, dioctadecyl disulphide has been tested in a 2-year oral dog study with 6000, 12000 and 24000 mg/kg test substance in the diet. Reduced body weight gains were observed in the high dose group of males compared to controls. Increases in haemoglobin concentration and in serum enzyme activities were observed in treated males and females at all dose levels at several time points, showing
an inverse dose-response relationship. The histopathological examination revealed deposits of the test compound in the liver, adrenals and lymph nodes in all treated animals, most notably in the low dose group and more marked in males. The Panel considered that this study confirmed the finding in rats of accumulation of the test substance in tissues. Again, however, due to absence of a positive dose-relationship for the observed effects and the presence of possible treatment-related effects at the lowest dose tested, the Panel considered that the results of the study could not be used for the purposes of risk assessment.

4.4. Reproduction/developmental oral study

In a reproduction/developmental toxicity study rats were fed with dioctadecyl disulphide at dietary levels of 0, 0.6, 1.2 and 2.4 % throughout pre-mating, mating, gestation and lactation periods. No parental effects were found with respect to general health, food consumption body weight development, fertility and reproductive performance. No effect was observed for litter size, litter sex ratio, and litter weight for all treated groups. No visible malformation was observed in pups. In conclusion, the NOAEL for reproductive toxicity in this study is at dose level of 2.4% or higher in the diet (corresponding roughly to ≥1200 mg/kg bw/day).

Accumulation aspects were not investigated in this study.

4.5. Toxicokinetic oral studies

Data from two toxicokinetic studies in male rats suggest that dioctadecyl disulphide was poorly absorbed from the gastrointestinal tract. Dose- and time-dependent concentrations in mesenteric lymph nodes and liver were very low. However, due to its very limited solubility the absorbed substance may deposit in mesenteric lymph nodes and other tissues after long-term administration. Due to the expected very limited capacity of the mammalian organism to dissolve such deposits and to metabolize the substance, there is a potential for accumulation of the test substance in specific tissues. This is supported by the findings of the chronic toxicity study in rats and the 2-year study in dogs.

DISCUSSION

Data from two toxicokinetic studies in male rats suggest that dioctadecyl disulphide was poorly absorbed from the gastrointestinal tract. Despite this low level of absorption, deposition of the substance in various tissues was observed, indicating a potential for accumulation.

Based on negative findings in five in vitro genotoxicity assays and one in vivo micronucleus assay dioctadecyl disulphide, the CEF Panel considered that there is no concern regarding the genotoxicity of dioctadecyl disulphide.

In chronic studies (one in rats and one in dogs) deposits of dioctadecyl disulphide were observed in several organs (e.g. liver, adrenals, lymph nodes) in rats and dogs along with increased organ weights and non-neoplastic and neoplastic findings in mesenteric lymph nodes of treated rats in all dose groups. Changes in haematology and serum enzyme activities were also observed in treated rats and dogs. Due to absence of a positive dose-relationship for the observed effects and the presence of possible treatment-related effects at the lowest dose tested (around 300 mg/kg bw/day in rats), no NOAEL for dioctadecyl disulphide could be derived. Therefore these studies could not be used for the purposes of risk assessment.

In a reproduction/developmental toxicity study the NOAEL for reproductive toxicity was at the highest administered dose (approximately 1200 mg/kg bw/day).

Based on the results from chronic studies, the Panel concluded that dioctadecyl disulphide can be deposited in various organs in two different species and therefore has a potential for accumulation.
CONCLUSIONS
The CEF Panel concluded that dioctadecyl disulphide is very poorly absorbed from the gastrointestinal tract, but is taken up to some extent as evidenced by the finding of insoluble test material in various organs. The substance therefore has a potential for accumulation, and to result in toxicity following chronic exposure. Due to uncertainties related to the dose-response relationship and the presence of possible treatment-related effects at the lowest dose tested in two species, the Panel could not derive a NOAEL for dioctadecyl disulphide from the available in vivo studies. Therefore the results from these studies could not be used for the purposes of risk assessment.

Therefore the CEF Panel does not concur with the TDI of 0.05 mg/kg/bw for dioctadecyl disulphide allocated by the SCF in 1995 and with the SML of 3 mg/kg food based on this TDI. However, considering the absence of genotoxicity, a limited daily intake of the substance up to 0.05 mg/person is unlikely to be a safety concern.

DOCUMENTATION PROVIDED TO EFSA
Dossier referenced: 49840. Dated: February 2011. Submitted by Clariant/Hoechst, containing unpublished reports as follows:
- six genotoxicity assays (five in vitro and one in vivo),
- two 90-day oral toxicity studies (one in rats and one in beagle dogs),
- two chronic oral studies,
- one reproduction/developmental toxicity study and
- two toxicokinetic studies.

REFERENCES

EC (European Commission), (2001). Guidelines of the Scientific Committee on Food for the presentation of an application for safety assessment of a substance to be used in food contact materials prior its authorisation; http://ec.europa.eu/food/fs/sc/scf/out82_en.pdf.
ABBREVIATIONS

AFC  Scientific Panel on additives, flavourings, processing aids and materials in contact with food
bw  body weight
CAS  Chemical abstracts service
CEF  Scientific Panel on food contact materials, enzymes, flavourings and processing aids
CHO  Chinese hamster ovary
Da  Dalton
DNA  Deoxyribonucleic acid
EC  European Commission
EFSA  European food safety authority
FCM  Food Contact Material(s)
LOAEL  Lowest observed adverse effect level
NOAEL  No observed adverse effect level
OECD  Organisation for economic co-operation and development
REF No  Reference Number
SCF  Scientific Committee on food
SML  Specific migration limit
TDI  Tolerable daily intake
UDS  Unscheduled DNA synthesis
w/w  Weight by weight