EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C2 - Management of scientific committees; scientific co-operation and networks

Scientific Committee on Food **SCF/CS/CNTM/CARGO/16 Final**

4 April 2003

Updated opinion of the Scientific Committee on Food on the potential risk to human health arising from the transport in ships' tanks of oils and fats from substances proposed as acceptable previous cargoes

(expressed on 4 April 2003)

B-1049 Bruxelles/ Brussel - Belgium
Telephone: switchboard 299.11.11. Fax: (+32-2) 299.4891

http://europa.eu.int/comm/food/fs/sc/scf/index_en.html

TERMS OF REFERENCE

The SCF is requested to update, if considered appropriate, Annex 2 of its opinion of September 1996 (substances submitted for assessment by the SCF but considered as not acceptable or provisionally acceptable at present as suitable substances for immediate previous cargoes to oils and fats for human consumption) in the light of new toxicological information, if available.

BACKGROUND

Chapter IV of the Annex to the directive on the Hygiene of Foodstuffs 93/43/EEC requires bulk liquid, granulate and powdered foods to be transported in receptacles and/or containers/tankers reserved for foodstuffs only.

The Commission and Member States recognised that the application of this requirement to the bulk transport of liquid oils and fats in ships would be impractical and could lead to unreasonable restrictions to world trade in these commodities. For these reasons, the Commission developed Directive 96/3/EC that derogates from certain provisions of Directive 93/43/EEC on the hygiene of foodstuffs. Commission Directive 96/3/EC allows for sea transport of fats and oils in bulk tanks which have previously been used to transport substances included in a positive list (list of acceptable previous cargoes).

Article 4 of Commission Directive 96/3/EC requires that it: "… shall be reviewed where one or more Member States, or the Commission consider that amendments are necessary in order to take account of scientific or technical developments. In any case the Annex shall be reviewed within one year of the adoption of this Directive.".

In its previous opinion of September 1996 (SCF, 1996) the SCF established a list of substances, which it considered as not acceptable or only provisionally acceptable at that time as suitable substances for immediate previous cargoes to oils and fats for human consumption.

Directive 96/3/EC was adopted before finalisation of the opinion of the SCF and therefore did not take account of the scientific advice, in particular the recommendations regarding certain substances considered as not (or provisionally) acceptable as immediate previous cargoes (Annex 2 of the SCF opinion).

The Commission therefore sought clarification on whether the conclusions of the SCF regarding these substances made in 1996 need to be updated. It asked the SCF to reconsider Annex 2 of its previous opinion in the light of new toxicological information, if available.

The Commission invited the Federation of Oils, Seeds and Fats Associations (FOSFA) to submit such information for consideration by the SCF. The Committee was requested to give priority to the substances that were "provisionally accepted" by SCF in 1996, i.e. iso-decanol, iso-nonanol, iso-octanol, MTBE, montan wax, petroleum wax and white mineral oils.

CRITERIA FOR THE RE-EVALUATION

For its previous opinion (SCF, 1996), the Committee considered that the acceptability of the substances in the list of acceptable previous cargoes be based on the following criteria: (SCF, 1996)

- no toxicological concerns, particularly with regard to their genotoxic and carcinogenic potential;

- efficacy of procedures used to clean ships' tanks between cargoes;

- dilution factor in relation to the potential amount of residue of the previous cargo and any impurity which the previous cargo might have contained and the quantity of oil or fat transported;

- subsequent application of refining processes and solubility relevant to the occurrence of possible contaminating residues;

- availability of analytical methods to verify the presence of trace amounts of residues or the absence of contamination of oils and fats.

For this evaluation the Committee has focused its attention on the toxicological properties of the substances on the list of acceptable previous cargoes for transport of bulk liquid oils and fats, without considering other aspects. Neither were the specifications of the transported oils and fats nor the purity of the previous cargo taken into account.

In considering the acceptability of the substances as previous cargoes the Committee noted that the Hygiene of Foodstuffs Directive 93/43/EEC requires all foodstuffs to be protected from the risk of contamination. In addition, it noted that in relation to the transport of foodstuffs where the conveyance and/or container has been used for the transport of anything other than foodstuffs, or for transporting different foodstuffs, Directive 93/43/EEC requires effective cleaning to be undertaken between loads to avoid the risk of contamination. The Committee also noted that the derogating directive 96/3/EC, allowing for the transport by sea of oils and fats in tanks not solely reserved for foodstuffs, includes a requirement for the captain of the vessel to keep accurate

documented evidence relating to the three previous cargoes and the effectiveness of the cleaning process between these cargoes.

SUMMARY OF THE EVALUATIONS

The re-evaluations were hindered by a lack of new data from industry. The Federation of Oils, Seeds and Fats Associations (FOSFA) submitted information on the substances to be re-evaluated in the period November 2001 - February 2002. An examination of the information provided by FOSFA revealed that most of the dossiers were far from complete to allow a sound scientific assessment of the toxicological properties of the substances that were given priority by the Commission. Depending on the completeness and adequacy of the information provided, the Committee followed a case-by-case approach in the reconsideration of the substances listed in Annex 2 of its opinion of 1996:

- In the case of 1,3-propanediol, cyclohexanol, cyclohexanone and methyl tertiary-butyl ether, the Committee searched for additional information and performed a re-evaluation, the results of which are presented in this opinion (see also the Table and the Annexes attached to this opinion).
- In other cases (i.e. 2,3-butanediol, iso-butanol, iso-decanol, iso-nonanol, iso-octanol, nonane, montan wax, paraffin wax and white mineral oils) the information available was considered inadequate or needed additional clarification. For these substances, the Committee was unable to complete new evaluations for this opinion and decided to maintain its previous opinion unchanged.

The conclusions of the considerations by the Committee have been summarised in Table A. A more detailed report of the evaluation of substances 1,3-propanediol, cyclohexanone/ cyclohexanol and methyl tertiary-butyl ether can be found in Annex I, II and III, respectively. A summary of these evaluations is given below.

1,3-Propanediol (*cf.* Annex I)

In the SCF's 1996 opinion on acceptable previous cargoes (SCF, 1996), 1,3-propanediol (1,3-propylene glycol), CAS Number 504-63-2, was considered not acceptable as a previous cargo because of inadequate toxicological data on a substance that is structurally of concern.
The only information submitted by industry on 1,3-propanediol in connection with previous cargoes was that it is soluble in water and has a boiling point of 210°C (letter from J Hancock to SCF Secretariat, dated 5 November 2001).

This substance was considered by the SCF in 1998 for use as a co-monomer in polyesters. Unpublished mutagenicity and developmental toxicity studies conducted between 1992 and 1995 were submitted in support of the application in food contact materials. On the basis of the mutagenicity and developmental toxicity data, the SCF recommended that the use of 1,3-propanediol was acceptable and should be classified in SCF List 3 (defined as substances for which an ADI or a TDI could not be established, but where the present use could be accepted) with a restriction of not more than 0.05 mg/kg of food. This opinion was adopted by the SCF at its 111th meeting in March 1998 (SCF, 1998). Since then, new information from a sub-chronic toxicity study has been published.

1,3-propanediol is of low toxicity following oral administration. In a 13-week rat study the NOAEL was 1000 mg/kg bw/day. In the developmental study, the LOAEL was 250 mg/kg bw/day for marginal fetal effects (retarded ossification).

In view of these data and provided that residues would be low after tank cleaning, the Committee considers that 1,3-propanediol can be accepted as a previous cargo.

Cyclohexanone and cyclohexanol (*cf.* Annex II)

In the SCF's 1996 opinion on acceptable previous cargoes (SCF, 1996), cyclohexanone, CAS Number 108-94-168, and cyclohexanol (hexahydrophenol), CAS Number 108-93-0, were considered not acceptable as previous cargoes because the data available at that time did not allow an adequate evaluation of the carcinogenicity and genotoxicity of either substance.

The only information submitted by industry was a limited data sheet on cyclohexanone (letter from J. Hancock to SCF Secretariat, dated 5 November 2001), without new toxicological data. A literature search in TOXLINE revealed three new studies published since the release of SCF's opinion in 1996: a case report in an adolescent, a human study on metabolism and toxicokinetics of cyclohexanol, and an oral study in rats on enzyme induction by cyclohexanol (see Annex II). There were however no new toxicological data that allow a re-evaluation of the carcinogenicity or genotoxicity of cyclohexanone and cyclohexanol. Therefore, the Committee considers its opinion of 1996 as unchanged and proposes that both compounds should not be accepted as previous cargoes.

Methyl tertiary-butyl ether (MTBE) (*cf.* Annex III)

In the SCF's opinion of 1996, MTBE, CAS Number 1634-04-4, was provisionally accepted as a previous cargo. Since then, a carcinogenicity study has become available. A comprehensive risk assessment report of the European Commission on MTBE has also been recently published (EC, 2002).

Based on the results of several *in vitro* and *in vivo* studies, MTBE is regarded as a non-genotoxic agent. The presently available data indicate that the tumours induced by MTBE in mice and rats are probably derived by a non-genotoxic mode of action and hence thresholds can be established for the toxic events triggering carcinogenesis.

The high water solubility of MTBE (48 g/L) allows effective cleaning of the cargoes by water washings at ambient temperature.

In view of these data and provided that residues would be low after tank cleaning, the Committee considers that MTBE can be accepted as a previous cargo.

REFERENCES

EC (European Commission) (2002). *Tert-butyl methyl ether.* European Union Risk Assessment Report, European Commission, Joint Research Centre, EUR 20417 EN (available at http://ecb.jrc.it/Documents/Existing-Chemicals/RISK_ASSESSMENT/REPORT/mtbereport313.pdf)

SCF (Scientific Committee for Food) (1996). *Opinion on the potential risk to human health arising from the transport in ships' tanks of oils and fats from substances proposed as acceptable previous cargoes.* Expressed on 20 September 1996. Annex VII to Document III/5693/96. DG III, European Commission, Brussels.

SCF (Scientific Committee for Food) (1997). *Methyl esters of fatty acids in previous cargoes.* Agenda item 15.3 of the Minutes of the 107th meeting of the Scientific Committee for Food, held on 12-13 June 1997 in Brussels. Document XXIV/1270/97-EN. DG XXIV, European Commission, Brussels.

TABLE A Summary of the evaluation of substances previously considered by the SCF (SCF, 1996) as not acceptable or provisionally acceptable as suitable substances for immediate previous cargoes to oils and fats for human consumption

Substance	CAS No.	Previous Evaluation (SCF 1996)	Present Evaluation (this opinion)
Cyclohexanol (hexahydrophenol)	108-93-0	**NOT ACCEPTABLE** The SCF has evaluated cyclohexanol in conjunction with cyclohexanone because of structural similarity and metabolic interconversion between cyclohexanol and cyclohexanone in vivo. There are no carcinogenicity data for cyclohexanol and limited mutagenicity data are equivocal with respect to clastogenicity.	**NOT ACCEPTABLE** No new toxicological information.
Cyclohexanone	108-94-1	**NOT ACCEPTABLE** The SCF considers that at this point in time there are inadequate data to fully evaluate these two substances but there are indications for concern regarding genotoxicity and carcinogenicity. For those reasons, the Committee is unable to endorse their inclusion in the list of acceptable previous cargoes.	**NOT ACCEPTABLE** No new toxicological information.

Substance	CAS No.	Previous Evaluation (SCF 1996)	Present Evaluation (this opinion)
Methyl esters of fatty acids: laurate palmitate stearate oleate	 111-82-0 112-39-0 112-61-8 112-62-9	**NOT ACCEPTABLE** Inadequate toxicological or technical data available. [NB. Considered **ACCEPTABLE AS PREVIOUS CARGO** at the 107th Plenary meeting (SCF, 1997).]	**ACCEPTABLE**
2,3-Butanediol (2,3-butylene glycol)	513-85-9	**NOT ACCEPTABLE** No toxicological data presented to the SCF. Limited technical data on ease of cleaning from the tank or removal by the oil refining process	**NOT ACCEPTABLE** Information provided was inadequate to reconsider SCF's opinion.
1,3-Propanediol (1,3-propylene glycol)	504-63-2	**NOT ACCEPTABLE** Inadequate toxicological data presented to the SCF on a substance which is structurally of concern.	**ACCEPTABLE** Opinion changed in view of new toxicological information and provided that residues would be low after tank cleaning.
iso-Butanol (2-methyl-1-propanol)	78-83-1	**NOT ACCEPTABLE** Limited toxicological data raises concern over its genotoxic and carcinogenic potential.	**NOT ACCEPTABLE** Committee is aware of number of issues that still need clarification.
Nonane	111-84-2	**NOT ACCEPTABLE** Inadequate toxicological or technical data available. No data presented to the SCF concerning the ease of cleaning from tanks or the ease of removal during the refining process.	**NOT ACCEPTABLE** Information provided was inadequate to reconsider SCF's opinion.

Substance	CAS No.	Previous Evaluation (SCF 1996)	Present Evaluation (this opinion)
iso-Decanol (isodecyl alcohol)	25339-17-7	**PROVISIONALLY ACCEPTABLE** No toxicological data, mixtures of isomers of uncertain composition. Easily removed by the oil refining process.	**PROVISIONALLY ACCEPTABLE** Information provided was inadequate to reconsider SCF's opinion.
iso-Nonanol (iso-nonyl alcohol)	27458-94-2		
iso-Octanol	26952-21-6		
Methyl tertiary butyl ether (MTBE)	1634-04-4	**PROVISIONALLY ACCEPTABLE** pending evaluation by SCF of studies concerning its use as an extraction solvent for food.	**ACCEPTABLE** Opinion changed in view of new toxicological information and provided that residues would be low after tank cleaning.
Montan wax	8002-53-7	**PROVISIONALLY ACCEPTABLE** Regarded as temporarily acceptable by the SCF as a food additive, highly insoluble	**PROVISIONALLY ACCEPTABLE** Information provided was inadequate to reconsider SCF's opinion.
Paraffin wax	8002-74-2 & 63231-60-7	**PROVISIONALLY ACCEPTABLE** only for those types which are considered temporarily acceptable as food additives by the Committee pending further data	**PROVISIONALLY ACCEPTABLE** Information provided was inadequate to reconsider SCF's opinion.
White mineral oils	8042-47-5	**PROVISIONALLY ACCEPTABLE** only for those types which are considered temporarily acceptable as food additives by the Committee pending further data	**PROVISIONALLY ACCEPTABLE** Information provided was inadequate to reconsider SCF's opinion.

ANNEX 1

1,3-Propanediol

BACKGROUND

In the SCF's 1996 opinion on acceptable previous cargoes (1), 1,3-propanediol (1,3-propylene glycol), CAS Number 504-63-2, was considered not acceptable as a previous cargo because of inadequate toxicological data on a substance that is structurally of concern.

The only information submitted by industry on 1,3-propanediol in connection with previous cargoes was that it is soluble in water and has a boiling point of 210°C (letter from FOSFA to SCF Secretariat, dated 5 November 2001). The LD_{50} in mice is 4773 mg/kg bw (2) and in rats is around 10g/kg (3). Other early research suggested that administration of 1,3-propanediol at 500 ppm in the diet of rats for up to 15 weeks caused cross-linking of DNA in liver and, to a limited extent, in testis (4). Liver but not testis homogenates were able to metabolise 1,3-propanediol to malondialdehyde, a reactive cross-linking agent.

This substance was approved by the SCF in 1998 for use as a co-monomer in polyesters and classified in List 3 (substance for which an ADI or TDI could not be established, but where the present use could be accepted) (5). Unpublished mutagenicity and developmental toxicity studies conducted between 1992 and 1995 were submitted in support of the application in food contact materials. Since then, new information from a sub-chronic toxicity study has been published.

EVALUATION OF THE DATA

Sub-chronic study in rats

Groups of 10 male and 10 female Crl:CD(SD)BR rats were given 1,3-propanediol orally by gavage at doses of 0, 100, 300 or 1000 mg/kg bw/day for 91 or 92 days (6). The study protocol conformed to US EPA Testing Guidelines (7) and was in compliance with EPA GLP Guidelines and OECD GLP. Body weights and food consumption were measured weekly. Blood was taken for haematology and serum chemistry at 4 and 13 weeks. Ophthalmoscopy was conducted at the start of the study and at 12 weeks. Measurement of organ weights and tissue pathology at termination were comprehensive. In addition to the usual parameters, spermatogenic analysis was also conducted at termination, which together with haematology would enable an assessment of toxicity produced by any DNA cross-linking.

There were no deaths prior to scheduled post-mortem. There were no treatment-related clinical signs and no effects on body weight or food consumption. There were no haematological effects, apart from a statistically significant reduction in white blood cell and/or lymphocyte counts in week 4 in all three groups of treated males. However, there was no dose-response relationship and the difference was attributable to a high value in the control group. There were some statistically significant changes in serum chemistry in females at 4 weeks (reduced AST at 300 and 1000 mg/kg bw/day, reduced cholesterol at 100 mg/kg bw/day and reduced chloride at 1000 mg/kg bw/day, increased glucose at all dose levels). However, the differences from controls were small, not dose-related and were not present at 13 weeks. At 13 weeks, total bilirubin was significantly reduced in females at 100 and 1000 mg/kg bw/day. There were no effects on serum chemistry at 4 or 13 weeks in males. There were no treatment-related effects on organ weights, gross pathology or microscopic pathology. There were no significant effects on testicular or epididymal sperm numbers, sperm production rate, motility or morphology.

The results of this study show no evidence of any systemic toxicity from administration of 1,3-propanediol up to 1000 mg/kg bw/day for 13 weeks. The study did not directly measure DNA cross-linking. However, it did not reveal any effects on the haematopoietic or spermatogenic systems, which might be expected to be susceptible to such effects. A NOAEL of 1000 mg/kg bw/day can be taken from this study.

Mutagenicity studies

Full reports of four mutagenicity studies on 1,3-propanediol were available to the SCF for its earlier review of 1,3-propanediol in the context of food contact materials (5). Summary data on these mutagenicity studies are also available from a submission by Degussa Corporation to the US EPA filed in accordance with Section 8(e) of the Toxic Substances Control Act (Substantial Risk Notification) (8).

1,3-propanediol was negative in a test for gene mutations in bacteria (5 strains of *Salmonella typhimurium*) and negative in a test for gene mutation in cultured mammalian Chinese hamster V79 cells *in vitro* (9,10). It did however show a clastogenic effect in a test for chromosome aberrations in Chinese hamster V79 cells *in vitro*, at the highest dose tested, in the absence but not in the presence of metabolic activation (11). When tested *in vivo* in a mouse micronucleus test, 1,3-propanediol was negative (12).

Developmental toxicity study

A conventional developmental toxicity study was conducted in the rat using dose of 0, 250 and 1000 mg/kg bw/day (13). The full study report was available to the SCF for its earlier review of 1,3-propanediol in the context of food contact materials (5). No adverse effects were observed

apart from reduced maternal weight gain on days 6-9 of gestation at 1000 mg/kg bw/day and retarded ossification in fetuses. The number of affected fetuses was significantly increased at 1000 mg/kg bw/day, with a marginal effect (LOAEL) at 250 mg/kg bw/day.

CONCLUSION

On the basis of the mutagenicity and developmental toxicity data, the SCF recommended that the use of 1,3-propanediol was acceptable and should be classified in SCF List 3 (defined as substances for which an ADI or a TDI could not be established, but where the present use could be accepted) with a restriction of not more than 0.05 mg/kg of food. This opinion was adopted by the SCF at its 111th meeting in March 1998.

1,3-propanediol appears to be of very low toxicity following acute or sub-chronic oral administration, with a NOAEL of 1000 mg/kg bw/day from the 13-week study and a LOAEL for marginal fetal effects (retarded ossification) of 250 mg/kg bw/day.

In view of these data and provided that residues would be low after tank cleaning, it is proposed that 1,3-propanediol can be accepted as a previous cargo.

REFERENCES

1. Scientific Committee for Food (1996). *Opinion on the potential risk to human health arising from the transport in ships' tanks of oils and fats from substances proposed as acceptable previous cargoes.* Expressed on 20 September 1996. Annex VII to Document III/5693/96. DG III, European Commission, Brussels.

2. Holman, N.M., Mundy, R.L. and Teague, R.S. (1979). Alkyldiol antidotes to ethylene glycol toxicity in mice. *Toxicol. Appl. Pharmacol.,* 49, 385-392.

3. Smyth, H.F., Seaton, J. and Fischer, L. (1941). The single dose toxicity of some glycols and derivatives. *J. Ind. Hyg. Toxicol.,* 23, 259-268.

4. Summerfield, F.W. and Tappel, A.L. (1984). Cross-linking of DNA in liver and testes of rats fed 1,3-propanediol. *Chem. Biol. Interactions,* 50, 87-96.

5. Scientific Committee on Food (1998). *Opinion on an additional list of monomers and additives for food contact materials.* Adopted on 19 March 1998. Annex II to Document

XXIV/1269/98. DG XXIV, European Commission, Brussels. (available at http://europa.eu.int/comm/food/fs/sc/scf/out10_en.pdf)

6. Gingell, R., Kirkpatrick, J.B. and Steup, D.R. (2000). Subchronic toxicity study of 1,3-propanediol administered orally to rats. *Int. J. Toxicol.,* 19, 27-32.

7. Code of Federal Regulations (1989). *EPA Toxic Substances Control Act Health Effects Testing Guidelines*. 40 CFR 798.2650.

8. Degussa Corporation (1996). Initial Submission - Letter from Degussa Corp to USEPA regarding genotoxicity tests of 1,3-propanediol, with attachments and dated 2/22/96. National Technical Information Service, US Department of Commerce, Springfield, VA 22161, USA. NTIS Order Number OTS0558466.

9. Degussa Corporation (1994). Degussa AG, unpublished report on Ames test. No. US-IT 94-0058-DGM, 1994.

10. Degussa Corporation (1994). Degussa AG, unpublished report on gene mutation test in cultured mammalian cells. No.US-IT 94-0060-DGM.

11. Degussa Corporation (1994). Degussa AG, unpublished report on chromosome aberration test in cultured mammalian cells. No. US-IT 94-0059-DGM, 1994.

12. Degussa Corporation (1995). Degussa AG, unpublished report on mouse micronucleus test. No. US-IT 94-0170-DGM, 1995.

13. *Examination of the influence of 1,3-propanediol, polymer grade, on the pregnant rat and the fetus by oral administration.* LPT report n.7413/92, 1992.

ANNEX 2

Cyclohexanone

BACKGROUND

In the SCF's 1996 opinion on acceptable previous cargoes (1), cyclohexanone, CAS Number 108-94-168, and cyclohexanol (hexahydrophenol), CAS Number 108-93-0, were considered not acceptable as previous cargoes because the data available at that time did not allow an adequate evaluation of the carcinogenicity and genotoxicity of both substances.

AVAILABLE DATA

Oral administration in the drinking water of cyclohexanone in one strain of mice and one strain of rats have given no convincing evidence of carcinogenicity. In mice B6C3F1 there was a slight increase of tumours that occur commonly in this strain, only at low dose. In rats F344 there was a slight increase of adrenal cortical tumours only in males treated with the low dose. Based on lack of epidemiological data and inadequate evidence in experimental animals the overall evaluation by IARC (1999) was: "Cyclohexanone is not classifiable as to its carcinogenicity to humans (Group 3)" (2).

Cyclohexanone did not induce gene mutations in bacterial cells (Ames test), whereas it induced gene mutations in Chinese hamster cells, chromosomal aberrations and ploidy changes in cultured human lymphocytes and in the bone marrow cells of rats treated *in vivo*. However, no conclusive evaluation of genotoxicity was possible. For cyclohexanol, the main metabolite of cyclohexanone, no carcinogenicity data are available. Limited genotoxicity data showed that cyclohexanol was inactive in the Ames test and unable to induce chromosomal aberrations in *Allium cepa*.

The only information submitted by industry was a limited data sheet on cyclohexanone (letter from FOSFA to SCF Secretariat, dated 5 Nov. 2001), without new toxicological data. It is poorly soluble in water and its boiling point is 156 °C. The oral LD_{50} in mice is 1400 mg/kg bw and in rats is 1535 mg/kg bw.

EVALUATION OF NEW DATA

A literature search in TOXLINE revealed the following three new studies: a case report in an adolescent, a human study on metabolism and toxicokinetics of cyclohexanol, and an oral study in rats on enzyme induction by cyclohexanol. These studies are reviewed below.

In vivo study on enzyme induction by cyclohexanol (3)

In order to characterise the previously described ability of cyclohexanol to induce CYP activity, the capacity of liver S9 from rats orally treated with 2.5% v/v cyclohexanol in drinking water for 5 days, to activate several carcinogenic nitrosamines into mutagens in *Salmonella typhimurium* TA100 test system was studied. Additionally, hepatic microsomes from the same treated-animals were analysed by western blot with specific antibodies again P450 protein families. Both studies showed an increase of CYP2E1 and CYP2B1/B2 cytochromes.

Metabolism and toxicokinetics of cyclohexanol in humans (4)

The metabolism and toxicokinetics of cyclohexanol and cyclohexane were studied in volunteers after 8-h periods of inhalation at concentrations of 1010 and 236 mg/m^3. Of the dose of absorbed parent compounds the yields of urinary cyclohexanol, 1,2- and 1,4-cyclohexanediol were 0.5%, 23.4% and 11.3% after exposure to cyclohexane, and 1.1%, 19.1% and 8.4% after exposure to cyclohexanol. The metabolic patterns of both compounds were very similar to that of cyclohexanone studied in the laboratory previously. For all three compounds, peak urinary excretion of cyclohexanol occurred at the end of the exposure period, after which it decayed rapidly.

A case report in humans (5)

A 15-year-old boy with no prior medical problems ingested cyclohexanone in a suicide attempt. The patient developed altered mental status, shock, metabolic acidosis, chemical hepatitis, and renal insufficiency. In addition, he developed rhabdomyelosis as evidenced by muscle pain, increased serum creatine phosphokinase levels and myoglobinuria. He was treated successfully with intubation, fluid resuscitation, dopamine and activated charcoal. The patient was discharged without clinical sequelae.

CONCLUSION

There are no new toxicological data that allow a re-evaluation of the carcinogenicity or genotoxicity of cyclohexanone and cyclohexanol. Therefore, the doubts on these aspects remain unresolved. On this basis, it is proposed that both compounds continue to be classified as "not acceptable" as previous cargoes.

REFERENCES

1. Scientific Committee for Food (1996). *Opinion on the potential risk to human health arising from the transport in ships' tanks of oils and fats from substances proposed as acceptable previous cargoes.* Expressed on 20 September 1996. Annex VII to Document III/5693/96. DG III, European Commission, Brussels.

2. IARC Monographs, Vol. 71, 1999.

3. Espinosa-Aguirre, J.J., Rubio, J., Lopez, I., Nosti, R. and Asteinza, J. (1997). Characterization of the CYP isozime profile induced by cyclohexanol. *Mutagenesis*, 12(3), 159-162.

4. Mraz, J., Galova, E., Nohova, H. and Vitkova, D. (1998). 1,2- and 1,4-cyclohexanediol. Major urinary metabolites and biomarkers of exposure to cyclohexane, cyclohexanone, and cyclohexanol in humans. *Int. Arch. Occup. Environ. Health,* 71(8), 560-565.

5. Zuckerman, G.B., Lam, S.C. and Santos, S.M. (1998). Rhabdomyolysis following oral ingestion of the hydrocarbon cyclohexanone in an adolescent. *J. Environ. Pathol. Toxicol. Oncol.,* 17(1), 11-15.

ANNEX 3

Methyl Tertiary-Butyl Ether (MTBE)

BACKGROUND

In the SCF's 1996 opinion on acceptable previous cargoes (1), methyl tertiary-butyl ether (MTBE), CAS Number 1634-04-4, was accepted only provisionally.

EXISTING DATA

A comprehensive risk assessment report of the European Commission on MTBE has been recently published (2).
MTBE is a gasoline octane enhancer approved in 1992 by the US EPA for use in motor gasoline and is one of the top 50 chemicals (by weight) produced in the USA. MTBE is also utilised to a lesser extent in Europe and other parts of the world.
Human exposure occurs via inhalation of car exhaust emission and from water contaminated with MTBE.
Because of the high production volume and the potential widespread human exposure the toxicity of MTBE has been extensively studied.

Metabolism

In vivo studies on the metabolism of MTBE in rats and in humans (3,4) indicate qualitatively similar overall metabolism.
MTBE is efficiently absorbed orally and via inhalation. Dermal absorption is moderate under occlusive conditions, whereas in open contact the uptake is strongly limited by the rapid evaporation. The extensive tissue distribution appears to be determined by solubility: concentrations in soft tissues are approximately the same as in blood, with the exception of fat that may be ten-fold higher.
MTBE is metabolised to formaldehyde and tert-butyl alcohol (TBA). Although in humans the biotransformation is mainly catalysed by CYP2A6 especially in the liver, in the rat other CYP enzymes seem to be involved, e.g. CYP2B1, CYP2E1 and CYP2A3 of the nasal epithelium. MTBE or its metabolites do not accumulate significantly in the human body (4).

Acute toxicity

The acute toxicity is low:
- rat: oral LD_{50} = 4000 mg/kg;
- mouse: oral LD_{50} = 5960 mg/kg.

Sub-acute and sub-chronic toxicity studies

- In a 28-day inhalation study (5) Fischer-344 rats and CD-1 mice were exposed to whole body MTBE vapour at 0, 400, 3000 and 8000 ppm five days/week, six hours/day. In the rats the only microscopic lesion that could be attributed to MTBE was the accumulation of protein in the kidney tubules in males at 3000 and 8000 ppm. Microscopically in mice high dose animals had hepatocellular hypertrophy in the centrilobular area, which was more severe than in the rats. For both rats and mice, a NOAEL of 400 ppm was obtained.

- In a 28-day oral study (6) Sprague-Dawley rats were exposed by oral gavage to MTBE at 0, 90, 440 and 1750 mg/kg bw/day five days/week. Pathology findings included hyaline droplet formation in proximal convoluted tubules of male rats in the mid- and high-dose groups; hyaline droplets were attributed to α2u-globulin. The NOAEL was 90 mg/kg bw.

- A 13-week, whole-body inhalation study with MTBE doses of 0, 250, 500 and 1000 ppm 5 days/week, 6 hours/day was carried out by Greenough *et al.* (7) on CD-rats. No treatment related deaths occurred during the course of the study. The only clinical sign reported was an increase in CNS depression with dose. Based on a change in female lung weight, a NOAEL of 500 ppm was derived.

- In a 13-week inhalation study (8) Fischer-344 rats were exposed to MTBE vapour concentrations of 0, 800, 4000 and 8000 ppm 5 days/week, 6 hours/day. No animals died. The only treatment-related clinical finding was ataxia in the 8000 ppm group. The analysis of the kidney slides showed the presence of α2u-globulin in the protein droplets found in tubules. Based on the effects seen in the male kidney, a NOAEL of 800 ppm was derived.

- In a 13-week oral study. Sprague-Dawley rats were administered MTBE daily by gavage 100, 300, 900, and 1200 mg/kg bw (9). Protein droplet nephropathy in the high dose group was two times more frequent than the controls. Males also had a significantly higher kidney weight. Based on the decrease in lung weight in females, a NOAEL of 300 mg/kg bw was derived.

Carcinogenicity

- In a two-year carcinogenicity study, groups of 50 Fischer-344 rats were exposed to MTBE concentrations of 0, 400, 3000 or 8000 ppm by inhalation for 6 hours/day, 5 days/week for 24 months (10). The high dose animals showed decreased body weight gain. Chronic progressive nephropathy increased mortality in all dose groups and was dose related. Renal tubular cell tumours were increased only in male rats at 3000 and 8000 ppm. The respective carcinoma rates were 0, 0, 6 and 0%: A dose dependent, statistical significant increase of testicular interstitial cell (Leydig cells) tumours was demonstrated as follows: 64% at 0 ppm, 70% at 400 ppm, 82% at 3000 ppm ($P<0.05$) and 94% at 8000 ppm ($p<0.05$) (10).

- Groups of CD-1 mice were exposed by inhalation at 0, 400, 3000 and 8000 ppm. The mortality rates in males ranged from 33% at 0 ppm to 49% at 8000 ppm in males; correspondingly, for females the mortality rates ranged from 27 to 33%. Hepatocellular hypertrophy was increased in the males at 3000 and 8000 ppm and in females at 8000 ppm. In females, the high dose group had a 20% incidence of hepatocellular adenomas compared with 4% incidence in controls (1/50 animal), which is within the historical control range of 0-4% (11).

- Sprague-Dawley rats were given, by gavage, 0, 250 or 1000 mg/kg bw of MTBE in olive oil, for 104 weeks (12,13). The test substance was administered on all days except on Wednesday and the weekends. The test animals had no treatment related clinical signs. Females had an increased incidence of lymphoimmunoblastic lymphomas and lymphoblastic leukaemia compared with the laboratory historical range (10%). In addition, in females, an increase of dysplastic proliferation of lymphoreticular tissues was observed in both dose groups. Dysplastic changes are occasionally associated with neoplastic transformation. The most frequently found neoplasm in both dose groups was lymphoimmunoblastic lymphoma localized in the lungs, with a proportion of >85% of the combined incidence. Male rats showed an increase in incidence of testicular interstitial cell adenoma. There were no signs of increase of testicular degeneration or atrophy. Statistically significant ($p<0.01$) increases were reported in Leydig cell tumours and lymphomas and leukaemia.

- *tert*-butyl alcohol (TBA), a metabolite of MTBE, increased the incidence of follicular-cell adenomas of the thyroid in female mice (14).

- MTBE did not show promoter activity after N-nitrosodiethylamine (DEN) initiation in B6C3F1-mice (15).

IARC (16) has concluded “there is inadequate evidence in humans” and “there is limited evidence in experimental animals”. According to the overall evaluation of the IARC “MTBE is

not classifiable as to its carcinogenicity to humans" (Group 3). (1998). An analogous classification was made by NTP (17).

Genotoxicity

MTBE was negative in several gene mutation tests in *Salmonella typhimurium* (2). William-Hill *et al.* (18) found a positive result in an Ames test using TA102 strain with and without metabolic activation. Addition of formaldehyde dehydrogenase (FDH) to the medium inhibited mutagenicity by 25-30%. In another study conducted by RBM (19) MTBE was not mutagenic in TA102 strain. MTBE was positive in a mouse lymphoma mutagenicity test only after metabolic activation suggesting formaldehyde involvement. However formaldehyde was generated outside the cell in an artificial environment (20). In a similar test *tert*-butyl alcohol (TBA) was negative (20).

MTBE did not induce gene mutations in Chinese hamster V79 cells (21,22). Two negative (22,23) and one positive (at the highest dose) (24) results were reported for *in vitro* UDS in rat hepatocytes. MTBE did not induce chromosome aberrations in Chinese hamster ovary cells (25) or micronuclei in NIH/3T3 cells (24). One equivocal and one negative result was obtained for in vitro SCE in Chinese hamster ovary cells (25).

In vivo, MTBE did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster* (26) and UDS in CD-1 mouse hepatocytes by inhalation (400, 3000 or 8000 ppm 2 days, 6 hours/day) (27). It did not induce gene mutations at the HPRT-locus of spleen lymphocytes in CD-1 mice treated by gavage (1, 10, 100 and 1000 mg/kg bw, 5 days/week for 3 days) (28). MTBE did not induce micronuclei in CD-1 mice erythrocytes by inhalation (400, 3000 or 8000 ppm 2 days, 6 hours/day) (29,30) or in Swiss Webster Mice erythrocytes by single i.p. injection (250, 500, 1000, 1500, 1750 mg/kg) (31).

MTBE did not induce chromosome aberrations in bone marrow of male Sprague-Dawley rats by i.p. route (0.04, 0.13 or 0.4 mg/kg, single dose or 3 doses/5 days) (32).
MTBE was positive in a Comet assay in lymphocytes of rats treated by gavage at single doses of 40, 400 or 800 mg/kg only at the highest dose (33).

Overall, despite minor shortcomings, based on the available information, MTBE cannot be regarded as a genotoxic agent. Based also on these data, the carcinogenic activity of MTBE in rodents was likely the result of a non-genotoxic mechanism.

Reproductive and developmental toxicity

- MTBE did not affect the reproductive function in rats exposed via inhalation in a single generation reproduction study in Sprague-Dawley rats at concentrations of 250, 1000 and 2500 ppm for 12 weeks, 5 days per week for 6 hours (34) nor in a two-generation study at concentrations of 0, 400, 3000 and 8000 ppm (35) that affected maternal food consumption.

- No significant developmental effects were seen in either mice or rats treated by inhalation up to 250 ppm (36).

- In one study in CD-1 mice treated by inhalation at 0, 1000, 4000 and 8000 ppm, increased incidences of post-implantation loss and cleft palate were seen at doses that also induced hypoactivity, ataxia and reduced food consumption in the dams (37). Another study in CD-1 mice conducted by inhalation at lower doses (up to 2500 ppm) that were less toxic to dams, did not provide evidence of developmental toxicity (36). NZW rabbits treated by inhalation at 1000, 4000 and 8000 ppm did not show signs of developmental toxicity even at the highest dose (37). The NOEL for maternal and developmental toxicity were both 1000 ppm in mice and 1000 ppm and 8000 ppm, respectively in rats.

Other effects on the endocrine and reproductive system

Most of the studies presently available are abstracts only. In summary, at high dose MTBE produces a number of effects in female mice. MTBE caused an increased metabolism of oestrogen in mouse liver, without affecting the level of the free hormone. MTBE seems to have a slight antioestrogen-like activity at very high doses. Consequently, a weight loss and morphologic changes were seen in the uterus. Oestrous cycle length and stages were also altered. Increased interstitial testosterone level was found in rats after a 28-day exposure (38). This study also reported decreased serum testosterone and LH. Corticosterone and aldosterone levels were elevated after continued exposure to high doses of MTBE. A clear decrease in serum corticosterone level was seen in the later phase of the chronic studies. Due to insufficient data, no NOAEL can be derived.

Neurotoxicity

In rats, MTBE produced signs of acute reversible CNS depression following exposure to 8000 ppm and, to a lesser extent, to 4000 ppm vapor. The NOAEL for these effects was 800 ppm. No persistent or cumulative neurotoxic effects were observed following exposure to MTBE at concentrations up to 8000 ppm for 13 weeks (8).

Other studies

Prescott-Mathews *et al.* (39) determined the ability of MTBE to cause α2u-globulin kidney nephropathy in male rat kidney. Male and female Fischer-344 rats were exposed to 0, 400, 1500 and 3000 MTBE vapour, six hours/day for 10 days. A clear positive correlation with MTBE dose and α2u-globulin-concentration was demonstrated.

CONCLUSION

The presently available data indicate that the tumours induced by MTBE in mice and rats probably derive by a non-genotoxic mode of action and hence thresholds can be established for the toxic events triggering carcinogenesis.

The high water solubility of MTBE (48 g/L) allows effective cleaning of the cargoes by water washings at ambient temperature.

In view of these data and provided that residues would be low after tank cleaning, the Committee considers that MTBE can be accepted as a previous cargo.

REFERENCES

1. Scientific Committee for Food (1996). *Opinion on the potential risk to human health arising from the transport in ships' tanks of oils and fats from substances proposed as acceptable previous cargoes.* Expressed on 20 September 1996. Annex VII to Document III/5693/96. DG III, European Commission, Brussels.

2. EC (European Commission) (2002). *Tert-butyl methyl ether.* European Union Risk Assessment Report, European Commission, Joint Research Centre, EUR 20417 EN (see http://ecb.jrc.it/Documents/Existing-Chemicals/RISK_ASSESSMENT/REPORT/mtbereport313.pdf)

3. Amberg, A., Rosner, E. and Dekant, W. (1999). Biotransformation and kinetics of excretion of methyl-*tert*-butyl ether in rats and humans. Toxicol. Sci., 51, 1-8.

4. Miller, M.J., Ferdinandi, E.S., Klan, M., Andrews, L.S., Douglas, J.F., and Kneiss, J.J. (1997). Pharmacokinetics and disposition of methyl t-butyl ether in Fischer-344 rats. *J. Appl. Toxicol.,* 17, S3-S12.

5. Chun, J.S., and Kintigh, W.J. (1993). *Methyl tertiary-butyl ether – 28-day vapor inhalations tudy in rats and mice.* Export, Pennsylvania: Bushy Run Research Centre, p. 387.

6. IITRI (1992). 28-day oral (gavage) toxicity study of methyl *tert*-butyl ether (MTBE). *G. Ital. Med. Lav. Ergon.,* 19, p. 63-65.

7. Greenough, R.J., McDonald, P., Robinson, P., Cowie, J.R., Maule, W., Macnaughtan, F. and Rushton, A. (1980). Methyl tertiary butyl ether (Driveron). *Three month inhalation toxicity in rats.* Edinburgh: Inveresk Research Intern., p. 227.

8. Daughtrey, W.C., Gill., M.W., Pritts, I.M., Douglas, J.F., Kneiss, J.J. and Andrews, L.S. (1997). Neurotoxicological evaluation of methyl tertiary-butyl ether in rats. *J. Appl. Toxicol.,* 17, S57-S64.

9. Robinson, M., Bruner, R.H. and Olson, G.R. (1990). Fourteen- and ninety-day oral toxicity studies of methyl tertiary-butyl ether in Sprague-Dawley rats. *J. Am. Coll. Toxicol.,* 9, p. 525-540.

10. Bird, M.G., Burleigh-Flayer, H.D., Chun. J.S., Douglas, J.F., Kneiss, J.J. and Andrews, L.S. (1997). Oncogenicity studies of inhaled methyl tertiary-butyl ether (MTBE) in CD-1 mice and F-344 rats. *J. Appl. Toxicol.,* 17, S45-S55.

11. Burleigh-Flayer .D., Chun, J.S. and Kintigh, W. I. (1992). Methyl-t-butyl ether: vapor inhalation oncogenicity study in CD-1 mice, Bushy Run Research Center, BRRC report 91N0013B. Submitted to the U.S. Environmental Protection Agency, under TSCA.

12. Belpoggi, F., Soffritti, M. and Maltoni, C. 1995. Methyl-tertiary butyl ether (MTBE) - a gasoline additive - causes testicular and lympho-haematopoietic cancers in rats. *Toxicol. Ind. Health,* 11, 119-149.

13. Belpoggi, F.., Soffritti, M. and Maltoni, C. 1998. Pathological characterization of testicular tumours and lymphomas-leukemias, and of their precursors observed in Sprague-Dawley rats exposed to methyl-tertiary butyl ether (MTBE). *Eur. J. Oncol.,* 3, 201-206.

14. Cirvello, J.D., Radovsky, J.E:, Heath, D.R., Farnell, D.R. and Lindamood, C. (1995). Toxicity and carcinogenicity of t-butyl alcohol in rats and mice following chronic exposure in drinking water. *Toxicol. Ind. Health.*, 11, 151-165.

15. Moser, G.J., Wolf, D.G., Wong, A. and Goldsworthy, T.L. (1997). Loss of tumour-promoting activity of unleaded gasoline in N-nitrosodiethylamine-initiated ovariectomized B6C3F1 mouse liver. *Carcinogenesis*, 18, 1075-1083.

16. IARC Monographs, 1999. Volume 73.

17. NTP (National Toxicology Program) (1998). *Technical Report on Carcinogenesis.*

18. Williams-Hill, D., Spears, C.P., Prakash, S., Olah, G.A., Shamma, T., Moin, T., Kim, L.K. and Hill, C.K. (1999). Mutagenicity studies of methyl-tert-butyl ether using the Ames tester strain TA102. *Mutat. Res.,* 446, 15-21.

19. RBM (1996). *Study of the ability of the test article MTBE to induce gene mutations in strains of Salmonella typhimurium.* Rome, Italy: Istituto di Ricerche Biomediche "Antoine Marxer" RBM S.p.A.

20. Mackerer, C.R., Angelosanto F.A., Blackburn, G.R. and Schreiner, C.A. (1996). Identification of formaldehyde as the metabolite responsible for the mutagenicity of methyl-t-butyl ether in the activated mouse lymphoma assay. *Proc. Soc. Exp. Biol. Ded.,* 212, 338-341.

21. Life Science Research (1989). *Gene mutation in Chinese hamster V79 cells. Test substance: MTBE.* P.51. Rome Toxicology Centre S.P.A.R.K.

22. Cinelli, S., Ciliutti, P., Falezza, A., Meli, C., Caserta, L., Marchetti, S., Seeberg, A.H. and Vericat, J.A. (1992). Absence of mutagenicity methyl tertiary-butyl ether. *Toxicol. Lett.* (Suppl.), 300, p. 36/10.

23. Life Science Research (1989). *Unscheduled DNA synthesis (UDS) in primary rat hepatocytes (Autoradiographic method).* P. 79. Rome Toxicology Centre S.P.A.R.K.

24. Zhou, W., Yuan, D., Huang, G., Zhang, H. and Ye, S. (2000). Mutagenicity of methyl tertiary-butyl ether. *J. Environ. Pathol. Oncol*., 16, p. 35-39.

25. Litton Bionetics Inc. (1980). *Mutagenicity evaluation of TBME A995 in an in vitro cytogenetic assay measuring sister chromatid exchange (SCE) and chromosome aberration frequencies in Chinese hamster ovary cells.* Kensington, Maryland: Litton Bionetics Inc.

26. Sernau, R.C. (1989). *Mutagenicity test on methyl tertiary butyl ether in Drosophila melanogaster sex-linked recessive lethal test.* Kensington, Maryland: Hazleton Laboratories America, Inc.

27. Vergnes, J.S. and Chun, J.J. (1994). *Methyl tertiary butyl ether: in vivo-in vitro hepatocyte unscheduled DNA synthesis assay in mice:* Bushy Run Research Centre.

28. Ward, J.D., Dalker, D.H., Hastings, D.A., Ammenhouser, M.M. and Legator, M.S. (1995). Assessment of the mutagenicity of methyl tertiary butyl ether at the HPRT gene in CD-1 mice. Society of Toxicology, Abstract 415.

29. Vergnes, J.S. and Kintigh,W.J. (1993). *Methyl Tertiary Buthyl Ether: Bone Marrow Micronucleus Test in Mice:* Bushy Run Research Centre.

30. McKee, R.H., Vergnes, J.S., Galvin, J.B., Douglas, J.F., Kneiss, J.J., and Andrews, L.S. (1997). Assessment of the in vivo mutagenic potential of methyl tertiary-butyl ether. *J. Appl. Toxicol*., 17, S31-S36.

31. Kado, N.Y., Kuzmicky, P.A., Loarca-Pina, G.L. and Huntaz, M.H. (1998). Genotoxicity testing of methyl tertiairy-butyl ether (MTBE) in the Salmonella microsuspension assay and mouse bone marrow micronucleus test. *Mutat. Res*., 412, 131-138.

32. Litton Bionetics Inc. (1979). *Mutagenicity evaluation of TBME 99% in rat bone marrow cytogenetic analysis*. Kensington, Maryland: Litton Bionetics Inc.

33. Lee, L.C., Quintana, P.J.E. and de Peyster, A. (1998). Comet assay evaluation of the effect of methyl t-butyl ether on rat lymphocytes. Society of Toxicology, Abstract 923.

34. Biles, R.W., Schroeder, R.E. and Holdsworth, C.E. (1987). Methyl-t-butyl ether inhalation in rats: a single generation study. *Toxicol. Ind. Health*, 3, 519-534.

35. Bevan, C., Neeper-Bradley, T.L., Tyl, R.W., Fisher, L.C., Panson, R.D., Kneiss, J.J. and Andrews, L.S. (1997b). Two-generation reproductive toxicity study of methyl tertiary-butyl ether (MTBE) in rats. *J. Appl. Toxicol*., 17, S13-S19.

36. Conaway, C.C., Schroeder, R.E., and Snyder, N.K. (1985). Teratology evaluation of methyl tertiary-butyl ether in rats and mice. *J. Toxicol. Environ. Health*, 16, p. 797-809.

37. Bevan, C., Tyl, R.W., Neeper-Bradley, T.L., Fisher, L.C., Panson, R.D., Douglas, J.F. and Andrews, L.S. (1997a). Developmental toxicity evaluation of methyl tertiary-butyl ether (MTBE) by inhalation in mice and rabbits. *J. Appl. Toxicol*., 17, S21-S29.

38. Williams, T.M.. Cattley, R.C. and Borghoff, J.J. (2000), Alterations in endocrine responses in male Sprague-Dawley rats following oral administration of methyl *tert* butyl ether. *Toxicol. Sci*., 54, p. 168-176.

39. Prescott-Mathews, J.J., Wolf, D.C., Wong, B.A. and Borghoff, S.J. (1997). Methyl *tert*-butyl ether causes alpha2u-globulin nephropathy and enhanced renal cell proliferation in male Fischer-344 rats. *Toxicol. Appl. Pharmacol*., 143, p. 301-314.