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COM/06/9

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Update on the Mutagenicity of Terephthalic Acid

Background

1. Terephthalic acid (TPA) (Figure 1) is used as a starting material in the manufacture of polyethylene terephthalate (PET). PET may be used to coat the internal surface and welded joints (side stripes) of food cans. PET can also be used to manufacture beverage bottles.

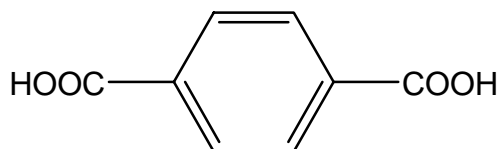


Figure 1. Terephthalic acid

2. TPA has been found to migrate at low levels from can coatings into food^[1]. In law, migration from can coatings is subject to the general restrictions applied to food contact materials, contained within the EC Regulation 1935/2004. The European Regulation is directly applicable in all Member States. The proposed Materials and Articles in Contact with Food (England) Regulations 2005 and similar regulations in the devolved administrations will provide for enforcement and new penalties that apply in the case of infringement of the Regulation. This European Regulation requires that articles intended to be brought into contact should not transfer their constituents to foodstuffs in quantities that could endanger human health or affect the nature or quality of the food. TPA is specifically controlled where it is used in plastic food contact materials and articles. Commission Directive 2002/72/EC, enacted Great Britain by The Plastic Material and Articles in Contact with Food Regulation 1998, as amended, stipulates that a specific migration limit (SML) for TPA of 7.5 mg/kg food or food simulant. TPA was included in the list of monomers studied in year 1 of the FSA funded survey on 'Chemicals used in plastic materials and articles in contact with food: compliance with statutory limits on composition and migration'^[2]. Fifty polyethylene terephthalate packaged foods were tested. There was no measurable migration of terephthalic acid.
3. The Scientific Committee on Food (SCF) reviewed the toxicology of TPA during the 1980's and established a temporary tolerable daily intake (t-TDI) of 0.125 mg/kg bw/day on the basis of the findings from a 90-day

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oral feeding study in rats. The t-TDI was temporary pending the submission of the full report of this study. The evaluation of terephthalic acid is now on the work programme of EFSA's Panel on food additives, flavourings, processing aids and materials in contact with food (AFC). This Panel is awaiting further information on terephthalic acid including reproductive toxicity and genotoxicity data.

4. TPA has been reported to have effects on bodyweight gain and effects on the bladder including formation of bladder calculi. The 90-day oral feeding study used by the SCF to set the t-TDI is unpublished and it is not possible to determine the dose levels at which these effects occurred or the NOAEL on which the t-TDI is based.

Previous Committee Evaluations

5. In October 2000, the Committee on Toxicity (COT) considered the health effects of TPA in the context of a survey on the migration of this compound from can coatings into food^[1]. The COT concluded that the concentrations of TPA that had been determined in foods analysed in the survey were not of concern for public health on the basis of the then available information. However, the COT requested that, in the light of the urinary tumours occurring in rats fed the highest dietary concentration of TPA in long-term studies, the view of the COM be sought on the potential *in vivo* genotoxicity of this compound^[3].
6. In November 2001, the COM considered the mutagenicity of TPA based on a limited data set. *In vitro* assays included several bacterial mutagenicity assays that, although finding TPA to be negative, were either poorly reported or had inadequate protocols^[4,5,6]. Overall, the Committee accepted that the evidence from the bacterial studies suggested that TPA is not mutagenic in a limited number of *Salmonella typhimurium* strains. An *in vitro* cytogenetics test in lung fibroblasts was also considered by the Committee^[7]. Although TPA was found to be negative when tested at a concentration of 2 mg/ml using an exposure period of 48 hours, the study did not address the influence of an exogenous metabolic activation system. In addition, the effect of shorter exposure periods were not investigated. Finally, members reviewed a negative *in vivo* micronucleus assay conducted with TPA in ICR mice^[8]. This was conducted to current standards but lacked toxicokinetic data and gave no direct measurement of bone marrow exposure. Signs of toxicity were reported which suggested that the test material had been absorbed into the systemic circulation and thus dose selection had been adequate.
7. The Committee considered that the limited *in vitro* mutagenicity data package and absence of toxicokinetic data in the *in vivo* micronucleus assay were insufficient to determine the mutagenic potential of TPA. Therefore, the Committee recommended that an adequately conducted *in vitro* cytogenetics test in mammalian cells was needed before any

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definite conclusions could be reached which would indicate that the bladder tumours in the rat carcinogenicity bioassay arose from a non-genotoxic mechanism ^[9]

8. In June 2003, a multi-generation reproductive toxicity study was evaluated by the COT, which concluded that dietary administration of 20 mg/kg diet TPA for two successive generations did not result in any alterations in reproductive performance ^[10]. However, histopathological changes in the urinary bladder and the kidney were reported at this dose. The COT considered it important to follow up these findings with further histopathological examination ^[11].
9. In March 2005, a report describing further histopathological examinations on the kidney and urinary bladders of animals in the TPA multi-generation study was submitted to the COT. This was accompanied by an expert report discussing histopathology of the kidneys of animals in this study, together with the previously conducted combined 90 day dietary toxicity study, one-generation reproduction study and two-year chronic dietary toxicity study in Fischer 344 rats.
10. A variety of changes were observed in the urinary bladder of animals of both sex receiving 20 mg/kg diet TPA. These changes comprised transitional epithelial hyperplasia, cystitis, inflammatory or mononuclear cell infiltration and haemorrhage. The incidence of observed changes was higher in the F1 generation than in F0 animals possibly reflecting the longer period of exposure of the former. The author of the expert report considered that these changes were related to treatment and indicated an irritant effect of the compound on the bladder mucosa at this dose level. No changes were observed in the bladder of animals receiving 1 and 5 mg/kg diet TPA or in controls ^[12,13].
11. The COT were satisfied with this analysis, determining a NOAEL of 425 mg/kg bw/day for this study, equivalent to the 5 mg/kg diet dose group. This did not indicate a need to reduce the temporary TDI of 0.125 mg/kg bw/day proposed by the SCF. However, the COT decided that a final statement should not be issued until the additional mutagenicity data on TPA had been evaluated by the COM ^[14].

Submitted Data

Metabolism Study in Mice (Annex A)

12. The test material for this study was prepared from unlabelled TPA (756.2 mg, 99.9% purity w/w, Lot HHAS116, BP Amoco Chemicals) mixed with [¹⁴C]-TPA (4.51 MBq = 0.25mg, 99.2% purity w/w, Amersham) in Aqueous carboxymethylcellulose (8.69g, 0.5% w/w). Seven groups of three male Crl:CD-1TM(ICR) BR mice were administered this test

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material (800 mg TPA/kg bw) via the intraperitoneal route. This dose, strain and administration route were selected to be consistent with those used in the mouse bone marrow micronucleus test (AA41MJ, Annex B), previously evaluated by the COM. Groups of mice were sacrificed at 2, 4, 6, 12, 24 and 48 h post treatment and the excreta of the 48 h dose group was collected for the duration of treatment.

13. The administered dose was extensively absorbed into the systemic circulation, widely distributed and rapidly excreted. Greater than 70% of the administered dose was excreted in the urine by 24 h. The highest mean tissue concentration was found in the kidney 2 h post administration (563 $\mu\text{g equiv/g}$, representing 1% of the administered dose) followed by bone (74 $\mu\text{g equiv/g}$). Isolation of murine femur bone marrow was difficult. Bone marrow could be extracted from two of the three mice in this dose group, with one below the limit of detection and the other 92 $\mu\text{g equiv/g}$, compared to blood (167 $\mu\text{g equiv/g}$) and plasma (221 $\mu\text{g equiv/g}$). Radioactivity declined rapidly in all tissues, with levels in most tissues below the limit of detection by 48 h.
14. Analysis of the urine found a single peak, which was reported to be a sulphate conjugate of TPA. The report offers the following evidence to support this analysis: *“The HPLC radiochromatograms of the pooled urine sample and cage wash contained a single, broad peak eluting at approximately 10 minutes. The negative mode mass spectra of this peak contained a base peak which was also the molecular ion [M-1] at 245 m/z. Although the HPLC retention time of this peak was very similar to that of the parent compound, the mass spectrum indicates that this peak is the sulphate conjugate of an acid moiety. The polarity of a free acid and a sulphate are very similar and therefore this metabolite was identified as the sulphate conjugate of terephthalic acid.”*
15. It should also be noted that there was considerable variation in tissue levels of TPA between the animals used within each dose group. This is particularly evident at 2 hours.

***In Vivo* Unscheduled DNA Synthesis (Annex C)**

16. A single 2000 mg/kg bw oral dose of purified TPA (BP Chemical N. V., Belgium, >99.9% purity w/w, Lot HAHS116) was assessed for its ability to induce unscheduled DNA synthesis (UDS) in the liver of male Alpk:AP_iSD rats. Groups of three rats were sampled at 2 and 16 h post administration. Assessment of the mean net nuclear grain counts and percentage of cells in repair indicate TPA did not induce UDS at either time point .
17. This study was performed to GLP, adhering to OECD guideline 486 (1997). Negative (vehicle) and positive (N-nitrosodimethylamine, 10 mg/kg bw) controls behaved as expected in this assay.

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***In Vitro* Cytogenetic Assay in Human Lymphocytes (Annexes D & E)**

18. Two *in vitro* cytogenetic assays were performed using human lymphocytes to assess the clastogenicity of TPA. In the first study (CTL/SV1255, Annex D), concentrations of 50, 250 and 500 µg/ml TPA (BP Chemicals N. V. 99.9% purity, Lot HAHS116) were applied in the presence and absence of S9 metabolic activation. This assay was limited to 500 µg/ml at which the pH of the culture medium was reduced from 7.10 to 6.74. At 1000 µg/ml the pH of the medium was reduced to pH 6.00.
19. Following the standard protocol, two independent experiments were performed; Experiment 1 assessed the clastogenicity of TPA following 3 h incubation in the presence and absence of S9 metabolic activation and Experiment 2 assessed TPA following 3 h incubation in the presence of S9 and 20h in the absence of S9. All cultures were harvested 20 h after dosing (68 h after culture initiation).
20. An apparent dose related reduction in mitotic index was observed in both experiments of this study. Statistically significant increases in the percentage of aberrant cells were observed following 20 h incubation in the absence of S9 metabolic activation ($p < 0.01$ at 250 and 500 µg/ml). In addition, there were small increases in the percentage of aberrant cells following 3 h incubation in the presence and absence of S9 metabolic activation. Therefore, under the conditions of the initial study (CTL/SV1255), TPA was found to be clastogenic.
21. A second study was commissioned (CTL/SV1318, Annex E) using sodium terephthalate (STP) obtained from a different supplier (Ex Avocado Research Chemicals, 99% purity w/w, Lot HAHS116). In this study, no reduction in pH was observed when tested up to 2100 µg/ml, the limit concentration for this assay (10 mM). Therefore, concentrations of 1000, 1500 and 2100 µg/ml were examined. As before, independent experiments assessed the clastogenicity of STP following 3 h incubation in the presence and absence of S9 metabolic activation and 3 h in the presence of S9 and 20h in the absence of S9. All cultures were harvested 20 h after dosing (68 h after culture initiation).
22. Small but statistically significant increases in the percentage of aberrant cells were observed, when compared to the vehicle control, following 3 h incubation in the presence and absence of S9 metabolic activation. These were not concentration related and were within the range of the historical control. Treatment did not appreciably reduce the mitotic index. No statistically significant increases in aberrant cells were observed in cultures incubated for 20 h in the absence of S9 metabolic activation. The author concluded that STP was not clastogenic under the conditions of this study.

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23. The study report for the initial study (CTL/SV1255) commented that the positive finding for TPA in this study should be taken in the context of the negative result for STP in the second study (CTL/SV1318). The author argues that the clastogenicity observed in the initial study was not associated with the terephthalate anion itself.
24. Both studies were performed to GLP, adhering to OECD guideline 473 (1997). Negative (vehicle) and positive (mitomycin C, 0.5 µg/ml; cyclophosphamide, 50 µg/ml) behaved as expected in both studies.

Questions for the Committee

25. The Committee is asked to comment on the information provided and to consider the following questions:
 - i) Does the Committee consider that the mouse metabolism study adequately supports the mouse micronucleus study, assessed by the COM in 2001 (Annex B)?
 - ii) Does the Committee agree with the conclusion that sodium terephthalate (STP) is not clastogenic when tested by *in vitro* cytogenetic assay in human lymphocytes?
 - iii) Does the Committee agree with the conclusion that, although terephthalic acid (TPA) is clastogenic when tested by *in vitro* cytogenetic assay in human lymphocytes, the second study assessing STP demonstrates that this clastogenicity is not due to the terephthalate ion itself ?
 - iv) Are the *in vitro* and *in vivo* genotoxicity assays sufficient to demonstrate that TPA does not have any significant mutagenic potential *in vivo* and thus indicate that the bladder tumours in the rat carcinogenicity bioassay are likely to arise by a non-genotoxic mechanism?

Annexes

Annex A: Terephthalic Acid: Mouse Metabolism Study (CTL/UM0853)

Annex B: Mammalian Erythrocyte Micronucleus Test, previously evaluated by the COM in 2001 (AA41MJ.123.BTL)

Annex C: Purified Terephthalic Acid: In Vivo Rat Liver Unscheduled DNA Synthesis Assay (CTL/SR1302)

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Annex D: Terephthalic Acid: In Vitro Cytogenetic Assay in Human Lymphocytes (CTL/SV1255)

Annex E: Sodium Terephthalate: *In Vitro* Cytogenetic Assay in Human Lymphocytes (CTL/SV1318)

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- ² Food Surveillance Information Sheet No. 43/03. (October 2001). Chemicals used in plastic materials and articles in contact with food. <http://www.food.gov.uk/multimedia/pdfs/fsis4303.pdf>
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- ⁸ Gudi, R. and Krsmanovic, L. (2001). Mammalian Erythrocyte Micronucleus Test. BioReliance Lab Study Number AA41MJ, 123.BTL (Annex B)
- ⁹ COM/02/S1 Statement on the Mutagenicity of Terephthalic Acid. (2001) <http://www.advisorybodies.doh.gov.uk/com/tpa.htm>
- ¹⁰ TOX/2003/37 Terephthalic acid: multi-generation reproduction toxicity study. <http://www.food.gov.uk/multimedia/pdfs/TOX-2003-37.pdf>
- ¹¹ Minutes of the meeting held on Tuesday 21 October 2003 in Conference Rooms 4 and 5, Aviation House.

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http://www.food.gov.uk/science/ouradvisors/toxicity/cotmeets/cot_2003/143212/cotmin21october2003

¹² TOX/2005/08 Terephthalic acid: multigenerational reproduction study additional histopathological examinations. <http://www.food.gov.uk/multimedia/pdfs/tox200508.pdf>

¹³ TOX/2005/15 Terephthalic acid: multigenerational reproduction study additional histopathological examinations. <http://www.food.gov.uk/multimedia/pdfs/TOX-2005-15.pdf>

¹⁴ Minutes of the COT meeting held on Tuesday 24 May 2005 in Conference Rooms 4 and 5, Aviation House, London. <http://www.food.gov.uk/multimedia/pdfs/cotfinalmin24may2005.pdf>

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Annex A

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

CTL/UM0853/REG/REPORT

Terephthalic Acid: Mouse Metabolism Study

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Annex B

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

AA41MJ.123.BTL

Mammalian Erythrocyte Micronucleus Test (previously evaluated by the COM in 2001)

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Annex C

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

CTL/SR1302/REGULATORY/REPORT

Purified Terephthalic Acid: *In Vivo* Rat Liver Unscheduled DNA Synthesis Assay

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Annex D

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

CTL/SV1255/REGULATORY/REPORT

Terephthalic Acid: *In Vitro* Cytogenetic Assay in Human Lymphocytes

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Annex E

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

CTL/SV1318/REGULATORY/REPORT

Sodium Terephthalate: *In Vitro* Cytogenetic Assay in Human Lymphocytes

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