

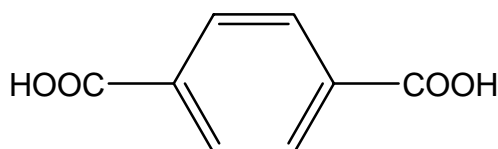
MUT/07/09

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

### Mutagenicity of Terephthalic Acid – Additional Cytogenetics Data

#### 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<sup>[1]</sup>. In law, migration from can coatings is subject to the general restrictions applied to food contact materials, contained within the EC Regulation 1935/2004. This European Regulation requires that articles intended to be brought into contact with food 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'<sup>[2]</sup>. In this survey, fifty polyethylene terephthalate packaged foods were tested, with no measurable migration of terephthalic acid into the food simulant.
3. In 1986, the Scientific Committee on Food (SCF) reviewed the toxicology of TPA and established a temporary tolerable daily intake (t-TDI) of 0.125 mg/kg bw/day on the basis bladder calculi formation and lack of body weight gain from a 90-day oral feeding study in rats. A t-TDI was established pending the submission of the full report of this study. A re-evaluation of terephthalic acid is now on the work programme of EFSA's Panel on food additives, flavourings, processing aids and materials in

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contact with food (AFC). This Panel is awaiting further information on terephthalic acid including reproductive toxicity and genotoxicity data from the studies considered by COT in 2005 and COM in 2006.

### Previous Committee Evaluations

4. 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<sup>[1]</sup>. 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<sup>[3]</sup>.
5. 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<sup>[4,5,6]</sup>. 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<sup>[7]</sup>. 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 nor investigate the effect of shorter exposure periods. Finally, members reviewed a negative *in vivo* micronucleus assay conducted with TPA in ICR mice<sup>[8]</sup>. This was conducted to current standards but lacked toxicokinetic data and provided 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 the dose selected had been adequate.
6. 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 definite conclusions could be reached which would indicate that the bladder tumours in the rat carcinogenicity bioassay arose from a non-genotoxic mechanism<sup>[9]</sup>.
7. In June 2003, a multi-generation reproductive toxicity study was evaluated by the COT, which concluded that dietary administration of 20 g/kg diet TPA for two successive generations did not result in any alterations in reproductive performance<sup>[10]</sup>. However, histopathological changes in the urinary bladder and the kidney were reported at this

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dose. The COT considered it important to follow up these findings with further histopathological examination <sup>[11]</sup>.

8. 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.
9. A variety of changes were observed in the urinary bladder of animals of both sex receiving 20 g/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 g/kg diet TPA or in controls <sup>[12,13]</sup>.
10. The COT were satisfied with this analysis, determining a NOAEL of 425 mg/kg bw/day for this study, equivalent to the 5 g/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 <sup>[14]</sup>.
11. In 2006 the COM reviewed data which had been submitted to address the concerns raised at the 2001 meeting. *In vitro* cytogenetics data was provided. An initial study using terephthalic acid was found to be positive following 20h incubation in the presence of exogenous metabolic activation by S9. Since pH was limiting in this study, a second study was conducted using sodium terephthalate. The use of the sodium salt of TPA did not affect the pH of the assay buffer, permitting testing up to the maximum concentration for this assay. Although the study author considered the assay to be negative, small but statistically significant increases in the percentage of aberrant cells were observed following 3 hours of incubation in the presence and absence of S9 metabolic activation. Members were concerned that a relatively small reduction of 1 pH unit could not fully account for the clastogenicity observed in the first study. Whilst the criteria for a positive response had not been fulfilled in the second study, the low incidence of aberrations in the control meant it was not possible to determine that terephthalic acid produced no effect. It was noted that 100 metaphases had been scored in the controls and at each dose group level. Members agreed that this should be increased to 200 to aid interpretation of the data.

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12. A mouse metabolism study was submitted to address concerns regarding bone marrow exposure in the mouse micronucleus study, which had been reviewed at the 2001 meeting. The Committee considered this study was not helpful in demonstrating bone marrow exposure; however, there was sufficient evidence of toxicity in the original micronucleus study to demonstrate systemic exposure. The findings of the micronucleus study were supported by a further *in vivo* study. Members agreed that this unscheduled DNA synthesis (UDS) study had been adequately conducted and was negative.
13. The committee considered that, overall, the evidence was consistent with a non-genotoxic mechanism for the bladder tumours seen in the rat carcinogenicity study; however, this conclusion would be reinforced if further investigation of the *in vitro* cytogenetics data was performed.

## **Submitted Data**

### ***In Vitro* Cytogenetic Assay in Human Lymphocytes incorporating additional metaphase counts (Annex A & B)**

14. Previously, an *in vitro* cytogenetics assay study was commissioned (CTL/SV1318) using sodium terephthalate (STP) (obtained from Avocado Research Chemicals, 99% purity w/w, Lot E24L42). 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). The study was 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.

15. As requested by the Committee, counts of an additional 100 metaphases (where possible<sup>†</sup>) were performed on the slides from SV1318. These additional counts are reported in Annex A (SV1380), with an overview report that combines the original counts with the additional counts in Annex B (SV1386).

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<sup>†</sup> See *number of cells examined* in table 4 of Annex A (pages 17 & 18)

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16. The combination of original and additional counts has resulted in slight variations in the frequency of aberrant cells throughout the study. Although statistically significant increases in aberrant cells persist, these remain within the historical range for the negative control.

### Questions for the Committee

17. The Committee is asked to comment on the information provided and to consider the following questions:
  - i) Is the committee now satisfied that the additional metaphase counts (SV1380) has reinforced the findings of the original study SV1318 ?
  - ii) Does the Committee now consider the two negative *in vivo* studies together with the available *in vitro* mutagenicity data are sufficient to conclude that terephthalic acid is not an in-vivo mutagen. Are members content with the previous conclusion agreed by COM that overall the evidence is consistent with a non-genotoxic mechanism for the bladder tumours seen in the rat carcinogenicity study?
18. A copy of the current COM statement on terephthalic acid is appended as Annex C. Members are asked to consider what revisions are required.

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## **References**

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- <sup>1</sup> Food Surveillance Information Sheet No. 7/00. (October 2000). Chemical Migration From Can Coatings into Food - Terephthalic and Isophthalic Acids. <http://www.food.gov.uk/science/surveillance/fsis2000/7phthal>
- <sup>2</sup> 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>
- <sup>3</sup> COT Statement on terephthalic and isophthalic acids from can coatings, <http://www.food.gov.uk/multimedia/pdfs/cotacids.pdf>
- <sup>4</sup> Brooks AL, Seiler FA, Hanson RL, Henderson RF. (1989) In vitro genotoxicity of dyes present in colored smoke munitions. *Environ Mol Mutagen* 13, 304-313.
- <sup>5</sup> Florin I, Rutberg L, Curvall M, Enzell CR (1980) Screening of tobacco smoke constituents for mutagenicity using the Ames' test. *Toxicology* 15, 219-232.
- <sup>6</sup> Zeiger E, Haworth S, Mortelmans K, Speck W (1985) Mutagenicity testing of di(2-ethylhexyl)phthalate and related chemicals in Salmonella. *Environmental Mutagen* 7, 213-232.
- <sup>7</sup> Ishidate M, Harnois MC, Safini T. (1988) A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. *Mutat Res* 195, 151-213.
- <sup>8</sup> Gudi, R. and Krsmanovic, L. (2001). Mammalian Erythrocyte Micronucleus Test. BioReliance Lab Study Number AA41MJ, 123.BTL (Annex B)
- <sup>9</sup> COM/02/S1 Statement on the Mutagenicity of Terephthalic Acid. (2001) <http://www.advisorybodies.doh.gov.uk/com/tpa.htm>
- <sup>10</sup> TOX/2003/37 Terephthalic acid: multi-generation reproduction toxicity study. <http://www.food.gov.uk/multimedia/pdfs/TOX-2003-37.pdf>
- <sup>11</sup> Minutes of the meeting held on Tuesday 21 October 2003 in Conference Rooms 4 and 5, Aviation House. [http://www.food.gov.uk/science/ouradvisors/toxicity/cotmeets/cot\\_2003/143212/cotmin21october2003](http://www.food.gov.uk/science/ouradvisors/toxicity/cotmeets/cot_2003/143212/cotmin21october2003)
- <sup>12</sup> TOX/2005/08 Terephthalic acid: multigenerational reproduction study additional histopathological examinations. <http://www.food.gov.uk/multimedia/pdfs/tox200508.pdf>
- <sup>13</sup> TOX/2005/15 Terephthalic acid: multigenerational reproduction study additional histopathological examinations. <http://www.food.gov.uk/multimedia/pdfs/TOX-2005-15.pdf>
- <sup>14</sup> 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/SV1380**

**Additional chromosome aberration analysis of slides from an in vitro cytogenetic assay on sodium terephthalate**

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## **Annex B**

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

**CTL/SV1386**

**Bridging Report to Combine the Data of an In Vitro Cytogenetic Assay in Human Lymphocytes and Additional Analysis of these Slides**

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