

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

MUT/07/24

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

Statement on the Mutagenicity of Terephthalic Acid

Background

1. Terephthalic acid (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. Terephthalic acid has been found to migrate from food contact materials at concentrations below 0.7 mg/kg food^[1, 2]. Migration from food contact materials is specifically controlled by European Regulation, which stipulates a specific migration limit (SML) for terephthalic acid of 7.5 mg/kg food.

Previous Committee Evaluations

3. In October 2000, the Committee on Toxicity (COT) considered the health effects of terephthalic acid 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 terephthalic acid 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 bladder tumours occurring in rats fed the highest dietary concentration of terephthalic acid (5% in the diet, equivalent to 2.5 g/kg bw/day) in long-term studies, the view of the COM be sought on the potential *in vivo* genotoxicity of this compound^[3].
4. In November 2001, the COM considered the mutagenicity of terephthalic acid based on a limited data set. *In vitro* assays included several bacterial mutagenicity assays that, although finding terephthalic acid 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 terephthalic acid is not mutagenic in a

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

limited number of *Salmonella typhimurium* strains. An *in vitro* cytogenetics test in lung fibroblasts was also considered by the Committee^[7]. Although terephthalic acid 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 terephthalic acid in ICR mice^[8]. This was conducted to current standards but lacked toxicokinetic data, so gave no direct measurement of bone marrow exposure. However, 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.

5. 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 terephthalic acid. 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^[9].
6. Subsequently the COT has evaluated a multi-generation reproductive toxicity study. Whilst dietary administration of up to 20 g/kg diet terephthalic acid for two successive generations did not result in any alterations in reproductive performance, histopathological changes in the urinary bladder and the kidney were reported at this dose^[10]. Further histopathological examination was conducted at the request of the COT. These changes comprised transitional epithelial hyperplasia, cystitis, inflammatory or mononuclear cell infiltration and haemorrhage. This expert report concluded that these treatment related changes indicated an irritant effect of the compound on the bladder mucosa at this dose level at 20 g/kg diet; however, no changes were observed in the bladder of animals receiving lower doses^[11,12]. 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. However, the COT decided that a final statement should not be issued until the additional mutagenicity data on terephthalic acid had been evaluated by the COM^[13].

Discussion of Submitted Data

7. In May 2006, the COM was presented with a submission of data that BP Chemicals Ltd had commissioned, following the 2001 meeting .

Mouse Metabolism Study

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

8. A mouse metabolism study was submitted to address concerns regarding bone marrow exposure at the doses selected for the mouse micronucleus study, which had been reviewed at the 2001 meeting. The test material for this study was prepared from unlabelled terephthalic acid (756.2 mg, 99.9% purity w/w) mixed with [¹⁴C]-terephthalic acid (4.51 MBq = 0.25mg, 99.2% purity w/w) in aqueous carboxymethylcellulose (8.69g, 0.5% w/w). Seven groups of three male Crl:CD-1TM(ICR) BR mice were administered this test material (800 mg terephthalic acid/kg bw) via the intraperitoneal route. This dose, strain and administration route were selected to be consistent with those used in the micronucleus study. 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 were collected for the duration of treatment.
9. 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 µg equiv/g, representing 1% of the administered dose) followed by bone (74 µg equiv/g). Difficulties in isolating murine femur bone marrow were experienced and bone marrow could be extracted from two of the three mice in the high dose group, with one below the limit of detection and the other 92 µg equiv/g, compared to blood (167 µg equiv/g) and plasma (221 µg equiv/g). Radioactivity declined rapidly in all tissues, with levels in most tissues below the limit of detection by 48 h. Analysis of the urine found a single peak, which was reported to comprise the parent compound and a sulphate conjugate of terephthalic acid (based on mass spectrometry data).
10. It was noted that there was considerable variation in tissue levels of terephthalic acid between the animals three animals used in each dose group, which was most apparent at 2 hours. Members also noted that the vehicle was different to the one that had been used in the micronucleus study, further complicating interpretation of the data.
11. The Committee considered that this metabolism study was not helpful in demonstrating target tissue exposure had been achieved in the micronucleus test. However, members noted there was evidence of systemic toxicity in the preliminary toxicity study and in the micronucleus study. Also, that there had been a reduction in the Polychromatic/Normochromatic erythrocyte ratio (P/N ratio) in the micronucleus test, which suggested that target tissue exposure to terephthalic acid, had occurred.

Unscheduled DNA Synthesis Study (UDS)

12. A second *in vivo* study was submitted to supplement the micronucleus study. A single oral dose of terephthalic acid (2000 mg/kg bw, >99.9% purity w/w) was assessed for its ability to induce UDS in the liver of male

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

Alpk:APfSD 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 terephthalic acid did not induce UDS at either time point. 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.

13. Members agreed that this unscheduled DNA synthesis (UDS) study had been adequately conducted and was negative.

In Vitro Cytogenetics Studies

14. As per the original COM data request, *in vitro* cytogenetics data using human lymphocytes to assess the clastogenicity of terephthalic acid were provided. In the first study, concentrations of 50, 250 and 500 µg/ml terephthalic acid (99.9% purity) 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.
15. Following the standard protocol, two independent experiments were performed; Experiment 1 assessed the clastogenicity of terephthalic acid following 3 h incubation in the presence and absence of S9 metabolic activation and Experiment 2 assessed terephthalic acid 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).
16. A 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 this initial study, terephthalic was found to be clastogenic.
17. A second study was submitted using the sodium salt of terephthalic acid, sodium terephthalate (99% purity w/w). In this study, no reduction in pH was observed when tested up to 2100 µg/ml, the maximum concentration stipulated by the protocol for this assay (10 mM). Therefore, concentrations of 1000, 1500 and 2100 µg/ml were examined. As before, independent experiments assessed the clastogenicity of sodium terephthalate 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).

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

18. 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 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 of the study report concluded that sodium terephthalate was not clastogenic under the conditions of this study. It was also argued that the positive finding for terephthalic acid in this study should be taken in the context of the negative result for sodium terephthalate in the second study. The author suggested that the clastogenicity observed in the initial study was not associated with the terephthalate anion itself.
19. 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.
20. 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 cultures 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.
21. These additional counts were provided for the May 2007 meeting. Members considered that the additional metaphase counts had reaffirmed the previous result, which suggested a weak clastogenic effect *in vitro*, and that the mechanism for this effect was unclear. Interpretation of this response was complicated by the incidence of aberrations in the control cells, which was much lower than the historical control. However, this study did not meet the criteria for a positive effect.

Conclusions

22. The Committee agreed that the two *in vivo* studies were adequate and negative, indicating that terephthalic acid is not an *in vivo* mutagen. The available evidence supported the previous COM conclusion of a non-genotoxic mechanism for the bladder tumours seen in the rat carcinogenicity study.

References

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

- ¹ 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>
- 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>
- ³ COT Statement on terephthalic and isophthalic acids from can coatings, <http://www.food.gov.uk/multimedia/pdfs/cotacids.pdf>
- ⁴ 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.
- ⁵ Florin I, Rutberg L, Curvall M, Enzell CR (1980) Screening of tobacco smoke constituents for mutagenicity using the Ames' test. *Toxicology* 15, 219-232.
- ⁶ 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.
- ⁷ 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.
- 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 TOX/2005/08 Terephthalic acid: multigenerational reproduction study additional histopathological examinations. <http://www.food.gov.uk/multimedia/pdfs/tox200508.pdf>
- ¹² TOX/2005/15 Terephthalic acid: multigenerational reproduction study additional histopathological examinations. <http://www.food.gov.uk/multimedia/pdfs/TOX-2005-15.pdf>
- ¹³ 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>