Opinion on Microcrystaline Cellulose (expressed on 19 th September 1997)

(N.B. Several References remain to be completed)

Terms of reference

To re-evaluate the safety in use of microcrystalline cellulose (MC) in the light of additional information received with respect to its general uses as set out in European Parliament and Commission Directive 95/2/EC.

Background

The Committee established in 1978 an ADI "not specified" for MC (1) but expressed a wish to be kept informed of any ongoing work to elucidate the problem of the persorption of ingested particulates which had been raised in connection with the safety assessment of MC. In 1990, the Committee agreed that the use of MC as an additive in weaning foods and gluten-free cereal based weaning foods was acceptable (2). In 1993 the Committee reconsidered microcrystalline cellulose in the light of the request to use material of particle size below 5µm in infant formula (3). The Committee remained concerned about the possibility of increased persorption in infants, considering the immaturity of the gut mucosa at that age, and its altered absorptive capacity in babies suffering from bowel disease, and withdrew its earlier acceptance of the use of microcrystalline cellulose in gluten-free weaning foods. It was unable to give any view on the safety of microcrystalline cellulose of particle size below 5µm as no toxicity data relevant to such material had been submitted. At that time the Committee also commented that a limit of 5µm should be introduced into the specification to ensure that only microcrystalline cellulose for which adequate toxicity data existed be permitted for food use. Further information has since been submitted and is considered here.

New information submitted

The new information on biochemistry included a study on 3 types of cellulose, administered orally to rats at 5% in their diet, which used glucose generation in vitro and in vivo as indicator of the digestibility of the celluloses tested. MC was found to be the least digestible of the 3 celluloses examined (4). Furthermore, a recent study in rats on the determination of the available energy from ingested MC and other incompletely digested carbohydrates was also supplied (45).

The earlier results of acute toxicity, irritancy and sensitisation studies were resubmitted (5-13) together with some recent similar studies on another MC preparation (47-52).

Genotoxicity was examined in several mutagenicity tests using different genetic endpoints. Three bacterial microsomal reversion tests, using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 with MC suspended in DSMO +/- S9 mix, produced no increase in revertants at doses up to 5000 µg/plate (14,15,53). Forward gene mutation tests in cultured L5178Y mouse lymphoma cells did not show any increase in mutants over a dose range of 100 - 1000 µg/ml (16,54). MC did not induce any unscheduled DNA synthesis in cultured primary hepatocytes up to doses of 1000 µg/ml. Some insolubility was noted with doses >5 µg/ml (17). Several in vivo micronucleus tests in mice showed no increase in micronuclei induced by MC doses up to 5000 mg/kg b.w. (18,55,56). MC was thus found to be non-genotoxic in a series of adequately performed mutagenicity tests using different genetic endpoints.

A recent study on subacute toxicity has also become available. A 28-day gavage study in Sprague-Dawley rats used doses of 1000, 2000, 3000, 4000 and 5000 mg/kg b.w./day in groups of 5 animals/sex. No adverse toxicological effects occurred at any of the dose levels tested. No persorbed particles of MC were detected in the gut or in the PeyersÂ’ patches at the highest dose level tested. The administered MC had a median particle size of 6 µm and contained 28% of

particles of size <5 µm (19).

Several new subchronic toxicity studies in Sprague-Dawley rats were also submitted. One of these was a 90-day feeding study using 25000 mg/kg feed and 50000 mg/kg feed of MC in the diet of the test animals. No adverse effects on body weight gain, haematological and clinical chemical parameters, organ weights of 6 major organs and the histopathology of 26 tissues including the GI tract, ileal lymphnodes and PeyersÂ' patches were noted. Inconsistent increases in food consumption occurred in both test groups. The NOAEL was 50000 mg/kg feed in the diet or approximately 4000 mg/kg b.w. as actually measured.. The MC tested had a median particle size of 21 µm but contained only 1% of material of a particle size <5 µm (20).

Another 90-day feeding study used 5% and 10% of MC in the diet. No adverse effects were noted on body weight gain, clinical chemical and haematological parameters, organ weights of 6 major organs and the histopathology of 33 tissues. Food consumption was increased dose-dependently. The NOAEL was 10% or approximately 6000 mg/kg b.w. as actually measured. The MC tested had a mean particle size of 32 µm but contained only 1% of material of a particle size <5 µm (21).

A further 90-day study used administration of MC by gavage and doses of 500 mg, 2500 mg and 5000 mg/kg b.w./day. No significant adverse effects were produced on survival, body weight gain, haematological and clinical chemical parameters, organ weights of 5 major organs, and the histopathology of 33 tissues. Only the high dose males showed reduced body weight gain, most probably the result of a nutritional effect. No specific pathological lesions were reported in spleen, gut wall and gut-associated lymphoid tissue (GALT). The NOAEL was 5000 mg/kg b.w./day. The MC tested had a median particle size of 6 µm and contained 28% of material of particle size <5 µm (22).

In a six months study groups of random bred rats of both sexes received either a control diet or a diet with 330 ppm of MC. At the end of this time six rats in each group were killed, their organs examined and tissues examined histopathologically. No adverse effects were observed (57).

Teratogenicity was examined in 2 studies using Sprague-Dawley rats. In one study MC was fed at doses of 25000 mg/kg feed and 50000 mg/kg feed in the diet from day 6-15 of gestation. No treatment-related adverse effects were noted on pregnancy, parturition and litter parameters. The NOAEL was 4410 mg/kg b.w. as determined from food consumption. The MC tested had a median particle size of 21 µm and only 1% of the material were particles of size <5 µm (23). The second study used 25000 mg/kg feed and 50000 mg/kg feed of another MC product in the feed from day 6-15 of gestation. No treatment-related adverse effects were seen on pregnancy, parturition and litter parameters. The NOAEL was 4589 mg/kg b.w. as determined from actual food consumption. The MC tested had a mean particle size of 32 µm and contained only 1% of material of particle size <5 µm (24).

The effects of cellulose fibre on tumour growth were investigated again by feeding artificial diets containing varied concentrations of either wheat bran or pure cellulose fibre to female F344 rats treated with i..v. 40 mg/kg b.w. N-nitrosomethylurea to induce mammary tumours. The wheat bran diet appeared to possess anti-promotion properties not observed with pure cellulose. The concentrations of serum oestrogens, urinary oestrogens and faecal oestrogens did not vary in a consistent, statistically significant manner (58).

The human clinical studies on various ingested MCs submitted were all concerned with changes of gastrointestinal function and nutrient balance and examined essentially faecal output, faecal composition, effects on blood biochemistry, the digestibility of the major nutrients and the bioavailability of essential micronutrients. Up to 30 g MC/day in the diet had no adverse effect on the function of the gastrointestinal tract, on haematological and on clinical chemical parameters except for the production of an increased faecal output (25, 35-39). These findings were supplemented by recent metabolic studies with MC (46) and with various cellulosic fibres (59,60).

Persorption aspects

Since the early publications in the 1960s on the persorption of ingested particulates and on the demonstration of their presence in the circulating blood stream, further research has been carried out which confirmed, that MC particles ranging in size from 5-150 µm could be persorbed and detected in venous blood samples taken 1-2 hours after ingestion

by rats, dogs, minipigs and in 1 human volunteer (26-28).

Further experiments, using i.v. administration to rats, showed some effects on haematology and renal function. MC particles could be identified in various tissues but these studies were of little relevance for assessing the biological significance of persorption following ingestion.. However, a combined one generation reproduction/chronic toxicity study in rats, in which the F1 generation was fed MC containing 90% of particles of size <20µm at 0, 3%, 10%, and 20% in their diet for two years, showed no adverse effects on litter parameters except some growth depression of the F1 weanlings at the top dose during the early growth phase only. Food consumption was increased in all MC-treated rats. After 12 months, MC particles were said to be detected in some organs and no microemboli were identified (Summary report only available).. Reports of some impairment of renal function without any associated histopathological changes and of some haematological changes in the highest dose group could not be confirmed in the surviving rats of the same study, which had been treated for a further year. (29,30). A more recent 90-day feeding study in rats, in which special precautions against contamination were taken, no MC particles were detected in any organ or tissue examined and no adverse histopathological effects were found. In particular, no kidney lesions were seen (22).

From the numerous studies reported in the literature it appears that persorption is a universal physiological process similar in mammals, the rat being a good model for man in this respect. Man and animals do not show accumulated particles in the intestines, or in the GALT, despite daily exposure to large numbers of persorbable particles in the diet throughout life. In recent appropriate studies, persorption has been shown to be an inefficient process. In single dose tests persorbed particulates are cleared from tissues within a few hours and they do not accumulate on repeated dosing even for several months (31). Interestingly, macrophages appear to be able to take up particles of size <2 µm while particles >16 µm do not appear to enter GALT (32). Some more recent studies using either biodegradable microspheres or other non-MC particulates confirmed uptake by GALT and systemic transfer to other tissues (40-44).

Reassessment of the techniques used in the early studies on persorption also revealed the need for taking meticulous precautions to avoid extraneous sample contamination, which could be misinterpreted as evidence for persorption. The absence of these precautions in the early studies therefore makes their results difficult to interpret. This point was examined specifically in a gavage study in rats using polystyrene particles under appropriate experimental conditions. The results confirmed intestinal persorption to be a very inefficient process in adult rats as only 0.05%-0.1% of the ingested particles could be recovered in the PeyersÂ’ patches (33). It should also be remembered, that many naturally occurring particulates are ingested frequently by man throughout life without causing any apparent harm(34).

Conclusions

This opinion applies only to general food uses of MC and does not apply to use in foods specially prepared for infants and young children including foods for special medical purposes for the same age group.

The additional toxicological information now submitted confirms the validity of the ADI "not specified" for MC previously established by the Committee. There is now evidence that MC has neither genotoxic nor teratogenic potential in the rat.

Early studies on the intestinal persorption of MC of varying particle size suggested that MC is persorbed, particularly if the particle size is <5 µm. This process is however very inefficient, at least in adult animals, and does not result in microembolic phenomena, nor does it appear to interfere with the immune function of the GALT. Recent studies on persorption in several species have shown that the rat provides an adequate model for this process in man. The two-year feeding study in rats showed no evidence of any histopathological or functional effects ascribable to accumulation of MC particles in any tissue as a consequence of persorption. The available human data on particles other than MC and animal studies on MC and the GALT suggest, that in normal adults, exposed over a comparatively short period, the intestinal persorption of MC of particle size even down to at least 5 µm would be unlikely to cause any adverse pathology in the gut and GALT. The Committee wishes to stress that there are no data available on the existence and the extent of persorption in very young animals or in human infants.

As a precautionary measure however, the Committee reiterates its view of 1993 (3) that the specification of MC should include a restriction on the content of material of particle size <5µm, The Committee is aware that a tolerance of 10%

by number of particles is achievable. Otherwise the CommitteeÂ's views on MC remain unchanged.

References

1. European Commission (1978) Report of the Scientific Committee for Food on Emulsifiers, Stabilisers, Thickeners and Gelling Agents. (Seventh Series). European Commission, Luxembourg.
2. European Commission (1991) First report of the Scientific Committee for Food on the essential requirements for weaning foods. Reports of the Scientific Committee for Food (Twenty-fourth Series). European Commission, Luxembourg.
3. Minutes of the 90th Meeting of the Scientific Committee for Food held on 16-17 September 1993 in Brussels. III/5481/93. European Commission DGIII/E/1.
4. Hsu,J.C.,Penner,M.H. (1989) Influence of cellulose structure on its digestibility in the rat. J. Nutr.,119,872-878.
5. FMC (1982a) Acute oral toxicity study in rats. Study no: 182-615 submitted to European Commission
   FMC (1991a) Acute oral toxicity study in rats. Study no: 191-1217 submitted to European Commission
6. FMC (1991b) Acute dermal toxicity study in rats. Study no: 191-1219 submitted to European Commission
   FMC (1982b) Acute dermal toxicity study in rabbits. Study no: 182-616 submitted to European Commission
7. FMC (1982c) Primary eye irritation study. Study no: 182-618 submitted to European Commission
   FMC (1991f) Primary eye irritation study in rabbits. Study no: 191-1218 submitted to European Commission
8. FMC (1982d) Primary skin irritation study. Study no: 182-617 submitted to European Commission
   FMC (1991c) Primary skin irritation study in rabbits. Study no: 191-1220 submitted to European Commission
9. FMC (1982e) Four hour acute dust inhalation toxicity study. Study no: 182-619 submitted to EU Commission
10. FMC (1991d) Skin sensitization study in guineapigs. Study no: 191-1216 submitted to European Commission
    FMC (1991e) Skin sensitization study in guineapigs. Study no: 191-1185 submitted to European Commission
11. Hazelton (1962a) Report submitted to European Commission
12. Hazelton (1962b) Report submitted to European Commission
13. Steege,H.,Phillipp,B.,Engst,R., et al. (1978) Microcrystalline cellulose powders. Properties and possible applications. Tappi,61,101-105
14. FMC (1991g) Salmonella/mammalian microsome plate incorporation assay. Study no: 191-1214 submitted to European Commission
15. FMC (1991h) Salmonella/mammalian microsome plate incorporation mutagenicity assay. Study no: 191-1188 submitted to European Commission
16. FMC (1991i) Mutagenicity test in the L5178Y TK $^{+/-}$ mouse lymphoma forward mutation assay. Study no: 191-1230 submitted to European Commission
17. FMC (1991j) Genotoxicity test in the assay for unscheduled DNA synthesis in rat liver primary cell cultures. Study no: 191-1229 submitted to European Commission
18. FMC (1991k) Mutagenicity test in vivo mammalian micronucleus assay. Study no: 191-1228 submitted to European Commission
19. FMC (1994) 28-day repeated oral dose range-finding study in rats. Study no: 194- 1847 submitted to European Commission
20. FMC (1991l) 90-day feeding study in rats. Study no: 191-1202 submitted to European Commission
21. FMC (1992a) 90-day feeding study in rats. Study no: 192-1711 submitted to European Commission
22. FMC (1995) 90-day repeated oral study in rats. Study no: 194-1926 submitted to European Commission
23. FMC (1991m) Teratology study in rats (dietary). Study no: 191-1213 submitted to European Commission
24. FMC (1992b) Teratology study in rats (dietary). Study no: 192-1712 submitted to European Commission
25. FMC (1995a) Additional documentation submitted to the European Commission, dated May 1995
26. Volkheimer,G. (1968) Zerebrale Gefäß-Verschlüsse durch Nahrungspartikel. Z. Geront., 1, 360-367
27. Pahlke,G. (1977) Zur Problematik der Füllstoffe in kalorienreduzierten Lebensmitteln. In: Bewertung von Risiken für die Gesundheit. Symposium Berlin 17-20.5.1976. Eds:Füllgraf und Ritzschel, Gustav Fischer Verlag, Stuttgart, pg 99-100
28. Pahlke,G.,Friedrich,R. (1975) Untersuchungen zur ernährungsmedizinischen Beurteil-ung von mikrokristalliner Cellulose. Mitteilungsblatt GdCh, 29, 67-70
29. Lewerenz,H.J.,Plass,R.,Bleyl,D.W.R. (1979) Untersuchungen zur Bewertung der Persorption von mikrokristalliner Zellulose im Warmblüter Organismus.-Wirkung mit dem Futter verabreichter MC bei Ratten.

Unpublished research report. ZIE, Potsdam-Rehbrücke
30. Lewerenz,H.J.,Bleyl,D.W.R.,Plass,R. (1981) Bericht über Untersuchungen im 2. Versuchsjahr kontinuierlicher Verabreichung von mikrokristalliner Zellulose mit dem Futter an Ratten. Unpublished research report. ZIE, Potsdam-Rehbrücke
31. Dayan,A.D. (1996) Occurrence and Significance of Persorption. Review prepared for FMC, dated 18.9.1996, submitted to European Commission.
32. LeFevre,M.C.,Hancock,D.C.,Joel,D.D. (1980) Intestinal barrier to large particulates in mice. J.Toxicol.Environ.Health,6,691
33. Simon,L.,Shine,G.,Dayan,A.D. (1994) Effect of animal age on the uptake of large particulates across the epithelium of the small intestine. Intern.J.Exper.Pathol.,75, 369-373
34. Dayan,A.D. (1995) The concept of persorption. Unpublished review prepared for FMC submitted to European Commission
35. Behall,K.M.,Scholfield,D.J.,Lee,K.,Powell,A.S.,Moser,P.B. (1987) Mineral balance in adult men: effect of four refined fibers. Amer.J.Clin.Nutr.,46,307-314
36. Bradlow,H.L.,Michnovicz,J.J.,Halper,M.,Miller,D.G.,Wong,G.Y.C.,Osborne,M.P. (1994) Long-term responses of women to indole-3-carbinol or a high fiber diet. Cancer Epidem.Biom.Preven.,3,591-595
37. Ismail-Beigi,F.,Reinhold,J.G.,Faraji,B.,Abadi,P. (1977) Effects of cellulose added to diets of low and high fiber content upon the metabolism of calcium, magnesium, zinc and phosphorus by man. J.Nutr.,107,510-518
38. King,J.C.,Costa,F.M.,Butte,N.F. (1982) Fecal mineral excretion of young men fed diets high in fiber components or phytate. Fed.Proc.,41,712
39. Niemi,M.K.,Keinanen-Kiukaanniemi,S.M.,Salmela,P.I. (1988) Long-term effects of guar gum and microcrystalline cellulose on glycaemic control and serum lipids in Type 2 diabetes. Eur.J.Clin.Pharmacol. 34,427-429
40. Eldridge,J.H.,Gilley,R.M.,Staas,J.K.,Moldoveanu,Z.,Meulbroek,J.A.,Tice,T.R. (1989) Biodegradable microspheres:vaccine delivery system for oral immunization. Curr.Top. Microbiol.Immunol. 146,59-66
41. Jani,P.U.,McCarthy,D.E.,Florence,A.T. (1994) Titanium dioxide rutile particle uptake from the rat GI tract and translocation to systemic organs after oral administration. Int.J.Pharmac. 105, 157-168
42. Jenkins,P.G.,Howard,K.A.,Blackhall,N.W.,Thomas,N.W.,Davis,S.S.,O'Hagan, D.T. (1994) Microparticulate absorption from the rat intestine. J.Contr.Rel. 29, 339-350
43. O'Hagan,D.T. (1990) Intestinal translocation of particulates - implications for drug and antigen delivery. Adv.Drug Deli.Rev. 5,265-285
44. O'Hagan,D.T. (1996) The intestinal uptake of particles and the implications for drug and antigen delivery. J.Anat. 289,447-482
45. Juhr,N.C.H.,Franke,J. (1992) A method for estimating the available energy of incompletely digested carbohydrates in rats. J.Nutr. 122,1425-1433
46. Walters,M.P.,Kelleher,J.,Findlay,J.M.,Srinivasan,S.T. (1989) Preparation and characterization of a (^{14}C)cellulose suitable for human metabolic studies. Br.J.Nutr. 62,121-129
47. FMC (1995a) Acute oral toxicity study in rats with Avicel AC-815. Study no: 195-2040 submitted to the European Commission
48. FMC (1995b) Acute dermal toxicity study in rats with Avicel AC-815. Study no: 195-2041 submitted to the European Commission
49. FMC (1995c) Acute inhalation study in rats with Avicel AC-815. Study no: 195-2045 submitted to the European Commission
50. FMC (1995e) Primary skin irritation study in rabbits with Avicel AC-815. Study no: 195-2043 submitted to the European Commission
51. FMC (1995d) Primary eye irritation study in rabbits with Avicel AC-815. Study no: 195-2042 submitted to the European Commission
52. FMC (1995f) Skin sensitization study in guinea pigs. Study no: 195-2044 submitted to the European Community
53. FMC (1995g) Mutagenicity test with Avicel AC-815 in the Salmonella-Escherichia coli/mammalian microsome reverse mutation assay with a confirmatory assay. Study no: 195-2047, conducted by Corning Hazelton, submitted to the European Commission
54. FMC (1994a) Mutagenicity test on Avicel CL-611 in the L5178Y TK+/- mouse lymphoma forward mutation assay with a confirmatory repeat. Study no: 194-1834, conducted by Hazelton Washington Inc, submitted to the

European Commission
55. FMC (1994b) Mutagenicity test on Avicel PH101 Pharmaceutical in an in vivo mouse micronucleus assay. Study no: 194-1837, conducted by Hazelton Washington Inc, submitted to the European Commission
56. FMC (1994c) Mutagenicity test on Avicel CL-611 in an in vivo mouse micronucleus assay. Study no:194-1835, conducted by Hazelton Washington Inc, submitted to the European Commission
57. Yartsev,N.M.,Ivanova,V.S.,Altymyshev,A.A.,Sarybayeva,R.I.,Vasil'kova,T.V. (1989) Anatomical and histological state of rats given microcrystalline cellulose in long-term experiments. Izvest.AN Kirogiz.SSR, 3,63-65
58. Cohen,L.A.,Zhao,Z.,Zhang,E.,Rivenson,A. (1996) Dose-response effects of dietary fiber on NMU-induced mammary tumorigenesis, estrogen levels and estrogen excretion in female rats. Carcinogenesis,17,45-52
59. Slavin,J.L.,Brauer,P.M.,Marlett,J.A. (1981) Neutral detergent fiber, hemicellulose and cellulose digestibility in human subjects. J.Nutr. 111,287-297
60. Livesley,G., Smith,T., Eggum,B.O.,Tetens,I.H., Nyman, M., Roberfroid, M., Delzenne, N., Schweizer,T.F.,Decombaz,J. (1995) Determination of digestible energy values and fermentabilities of dietary fibre supplements: A European interlaboratory study in vivo. Br.J.Nutr. 74,289-302