Data on Residual Content of Substance in the FCM

In this guide

In this guide

Data on Residual Content of Substance in the FCM

Actual Content

89. Calcium tert-butylphosphonate does not react with LDPE, nor is it volatile, therefore, the amount of additive incorporated into the polymer is the actual content expected to be present in the test materials. Because there is no expectation of loss of calcium tert-butylphosphonate, the presence of the additive at the intended use level in the actual test material used in migration experiments was not determined by analytical data.

Microbiological Properties of the Substance

90. This section is not applicable, as there are no microbiological considerations when calcium tert-butylphosphonate is used as intended. Calcium tert-butylphosphonate is neither intended nor expected to function as an antimicrobial.

Toxicological Data

91. The toxicological data for calcium tert-butylphosphonate are presented below.

Genotoxicity

Bacterial Reverse Mutation Assay

92. The study was conducted in accordance with test guideline OECD no.471.

93. Salmonella typhimurium strains TA1535, TA1537, TA98, and TA100 and Escherichia coli strain WP2 uvrA were exposed to calcium tert-butylphosphonate at concentrations ranging from 1.5 –5,000 µg/plate with and without metabolic activation. Dimethyl formamide was selected as the vehicle in both studies.

94. The first experiment used the plate incorporation method, and the second experiment used the preincubation method. Both assays were performed in the absence and presence of metabolic activation by phenobarbital/β-naphthoflavone-induced rat liver S9 fraction (S9-mix).

95. Six concentrations in the range of 15–5000 µg/plate were applied in the second experiment, while the test item was tested at eight concentrations ranging from 1.5 to 5000 µg/plate in the first experiment. On triplicate plates, all concentrations were assessed along with positive and negative (vehicle) controls.

96. A test item precipitate (white and granular in appearance) was noted in both the presence and absence of the metabolic activation system (S9-mix) at 5000 and from 1500 µg/plate in Experiments 1 and 2, respectively.

97. Results for the negative controls (spontaneous mutation rates) and viability were acceptable, both with and without metabolic activation. All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies, both with and without metabolic activation as appropriate. Thus, the sensitivity of the assay and the efficacy of the S9-mix were validated.

98. Under the conditions of this study, the test item did not induce an increase in the frequency of revertant colonies that met the criteria for a positive result, either with or without metabolic activation (S9-mix). Under the conditions of this test, the test item was considered to be non-mutagenic.

In Vitro Micronucleus Test in Human Lymphocytes

99. The study was conducted in accordance with test guideline OECD No. 487.

100. Duplicate human peripheral blood lymphocytes were used in the in vitro micronucleus assay to evaluate calcium tert-butylphosphonate (purity 98.4%). The test was conducted in accordance with GLP guidelines. The methodology for the cytokinesis block micronucleus assay was used. Demecolcine, mitomycin C, and cyclophosphamide served as positive controls. The test item was suspended in DMSO since it was insoluble in the culture medium. Liver S9 from rats induced by phenobarbital and β-naphthoflavone was applied to duplicate lymphocyte cultures from a healthy donor for 4 hours, either in the presence of cytochalasin B (CytB) for 24 hours after recovery, or for 24 hours without S9 and then for a further 24 hours with CytB. In every experiment, concentrations of 50, 100, and 200 µg/mL test item were used.

101. The concentration of 200 µg/mL was the lowest precipitating concentration in all three exposure groups and the maximum concentration selected for evaluation of micronuclei in the binucleate cells.

102. For the test item and the positive controls, micronuclei were scored in 2000 binucleated cells per concentration (1000 from each culture), and in 4000 binucleated cells (from 4 cultures) for the vehicle controls. A toxicity index known as the Cytokinesis-Block Proliferation Index was calculated using 500 cells per culture. Under no experimental circumstance with the test item, did treated cultures show any increase in binucleated cells containing micronuclei or dose-related toxicity as compared to vehicle controls.

103. The criteria for a negative result were therefore achieved in all three of the exposure groups.

104. Whilst evaluating the toxicological data supplied by the Applicant, the FCMJEG sought clarification as to why the solvent that was used differed between the Ames test and the in vitro micronucleus test (DMF vs DMSO). Further information on the solvent selection was submitted by the Applicant, which the FCMJEG considered sufficient.

105. It was concluded that the test item, calcium tert-butylphosphonate, did not induce any statistically significant increases in the frequency of binucleate cells with micronuclei in either the absence or presence of a metabolising system. Calcium tert-butylphosphonate treatment did not show marked cytotoxicity.

106. The results from all three exposure conditions in the main experiment indicated that the frequency of cells with micronuclei was within the normal range in negative controls and significantly increased in positive controls compared to the historical data.

107. The test item was therefore considered to be non-clastogenic and non-aneugenic to human lymphocytes in vitro.

Overall summary of genotoxicity studies

108. The available toxicology data, therefore, showed calcium tert-butylphosphonate to be negative in the in vitro Ames test and in vitro micronucleus (MN) assay and therefore unlikely to be of concern for genotoxicity, especially given its low exposure in humans.

Other considerations

109. EFSA has published guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age (EFSA, 2017). In this age group, infant formula or human milk may be the only source of nutrition and, health-based guidance values for the general population do not apply without further considerations. Organ development and the absorption and distribution rates of a substance in infants and adults may differ. Risk assessment should be on a case-by-case basis, depending on whether the substance is added intentionally to food and is systemically available.

110. The FCMJEG noted that calcium tert-butylphosphonate was not intentionally added to food, however it is an organophosphate compound, and such compounds can cause neurotoxicity. If migration and absorption occur, the extent of any transport across the blood brain barrier is unknown, which then, may or may not lead to potential neurotoxicity in infants. Although the potential for exposure is probably very low, contact with infant formula and infant milk could not be evaluated. The main concern is the lack of data in this potentially sensitive age group, and that infants <16 weeks are expected to be exclusively fed on breast milk and/or bottle-fed infant formula, with potential exposure to the FCM from bottles.

111. The FCMJEG noted that if the Applicant wishes to submit further data to aid in the assessment of the <16-week infant group then an extension of use application would need to be submitted.