Opinion Paper

Joint Expert Group on Food Contact Materials

Committee Advice Document on the safety of 2-hydroxyethyl methacrylate phosphate as a monomer for use in the manufacture of plastic food contact materials and articles

Joint Expert Group on Food Contact Materials (FCMJEG)
Science, Evidence and Research Directorate
Regulated Products Dossier Assessment
ID: RP1190
Assessment finalised: 21/06/2024

Summary

1. A request was received for the Joint Expert Group on Food Contact Materials (FCMJEG) to provide a risk assessment on the safety of 2-hydroxyethyl methacrylate phosphate (HEMAP), as a monomer in a commercial product for use in the manufacture of kitchen countertops and sinks that are intended for contact with all types of food.

2. All components of the commercial product are listed in assimilated Regulation EU No. 10/201 on plastic materials and articles intended to come into contact with food. The application and the following assessment are for HEMAP only, not the commercial product.

3. Satisfactory information regarding the identity of substance, physical and chemical properties, intended application of substance, data on migration of substance and toxicological data were submitted.

4. The specific migration of the sum of HEMAP plus its phosphate and diphosphate esters under the worst foreseeable conditions of use was 24.8 µg/6 dm^2 (assumed that this is equivalent to contact with 1 kg food).

5. The toxicological information that formed the basis of the risk assessment was a bacterial reverse mutation test (Ames test), which was conducted in accordance with OECD No. 471, and an *in vitro* mammalian micronucleus test, in accordance with OECD No.487, on the commercial product. Results of the Ames test and *in vitro* micronucleus (MN) test showed no mutagenic, clastogenic or aneugenic potential for the commercial product under the experimental conditions described.

6. Taking into account that the specific migration of the sum of HEMAP plus its phosphate and diphosphate esters is not expected to exceed 50 µg/kg food and the negative results in the Ames and in vitro micronucleus tests, the

FCMJEG proposed a specific migration limit (SML) of 0.05 mg/kg food for HEMAP.

7. Overall, the FCMJEG considered the information and data provided were sufficient to conclude that there was no concern for a risk to human health from the use of HEMAP in the specific final commercial mixture in the manufacture of kitchen countertops and sinks up to a maximum percentage in formulation of 0.35%.

Introduction

8. An application was submitted to the Food Standards Agency (FSA) regarding the use of 2-hydroxyethyl methacrylate phosphate (HEMAP) as the major component in a commercial product to be used in the manufacture of kitchen countertops and sinks intended for contact with all types of food.

Dossier contents/Discussion

Identity and manufacturing details (starting substances)

9. 2-Hydroxyethyl methacrylate phosphate (HEMAP) is manufactured by mixing multiple starting substances, including 2-hydroxyethyl methacrylate (HEMA).

10. The following substances (that make up HEMAP) are formed as successive mono-, di-, and tri-hydroxyethyl methacrylate esters, on the phosphate or diphosphate cores:

- Mono-substituted phosphoric acid
- Di-substituted phosphoric acid
- Tri-substituted phosphoric acid
- Substituted diphosphates.

11. Reviewing the information provided, the FCMJEG agreed that no by-products were anticipated to form in the production of HEMAP but that any impurities/substances detected were residuals of the starting substances.

Physical and chemical properties

12. As HEMAP is never separated from the commercial product, the physical and chemical properties presented below are for the commercial product and not HEMAP itself.

13. The final commercial product has a freeze and melting point below -80°C, and a decomposition temperature of 225°C.

14. The final commercial product is not soluble in water but has good solubility in ethanol and olive oil (greater than 1% at 25°C).

15. The following values were estimated for the log Po/w

- Mono substituted phosphoric acid: -0.03
- Di substituted phosphoric acid: 1.2
- Tri substituted phosphoric acid: 2.4
- Substituted diphosphoric acid: in the range of -1.03 and 2.67

Reactivity

16. Considering all available information, the FCMJEG agreed that under the foreseeable conditions of use, the substance was not expected to be reactive once incorporated into the countertop. Furthermore, no hydrolysis, unintentional decomposition/transformation or interactions with food substances were expected.

Food contact material

17. HEMAP is used in the production of the polymer fraction of acrylic counter tops and sinks. The maximum percentage in formulation is 0.35% of HEMAP.

18. As such, HEMAP can come into contact with all types of food prepared in kitchens resulting in the potential for migration from the countertops manufactured with the substance.

19. It is expected that the countertops and sinks would be cleaned before each use, including initial use.

Data on migration of substance

Contact time and temperature

20. The migration test samples were sheets of solid surface acrylic countertops that were fabricated both with and without the final commercial mixture, containing HEMAP.

21. The duration of direct contact with food is not expected to exceed several hours (48 hours as a worst case) at room temperature (RT, approximately 20-25°C), or a short time (e.g., 1 hour) at high temperature (up to 70ºC), followed by cooling and additional contact time at RT, unlikely to exceed 2 hours. Only repeated use applications were considered.

22. Hot baked goods or cooked meats are not typically expected to be placed in direct contact with acrylic countertops. However, this exposure scenario was used for the migration study and therefore represented a foreseeable worst-case. The standard contact area was assumed to be 6 dm^2 per kg food. Foods present an extractive medium into which the countertop components may migrate. Under normal conditions of use foods would be expected to be left on countertops or sinks only for very short periods of time prior to consumption. In practice, foodstuffs are generally in or on another

article (e.g., fruit basket or chopping board). The FCMJEG agreed that these conditions of testing would be highly exaggerative and would therefore be reflective of a conservative model of the intended use.

23. Assimilated Regulation EU No. 10/2011 requires that compliance for repeated-use food-contact articles like countertops shall be confirmed on the basis of the level of the migration found in the third exposure carried out on a single sample using a fresh portion of simulant for each exposure.

24. Here, single exposures were conducted for 10 days (240 hours) at 40°C using 10% ethanol and 3% acetic acid, as the aqueous and acidic food simulants, respectively. A single exposure for 2 days at 20°C using 95% ethanol was used as an alternative to vegetable oil, as the fatty food simulant. The FCMJEG accepted that, although three successive exposures were not carried out, the exposure conditions here were more severe and exaggerative of the intended use.

Analytical method for the determination of the specific migration into food simulants

25. The precision of the test method was determined by calculation of the coefficient of variation (CV%) using the mean calculated analyte concentration divided by the standard deviation obtained from the recovery experiment that was performed five-fold.

26. The recovery was acceptable for all food simulants with mean recovery and coefficient of variation of:

- 10% ethanol: 98% (Bias 2%) CV 3%
- 3% acetic acid: 96% (Bias 4%) CV 4%
- 95% ethanol: 99% (Bias 1%) CV 3%

Specific migration

27. The applicant used the standard assumption that 1 kg food is in contact with 6 dm^2 of food contact material (FCM). An overview of the obtained migration results for the commercial product with and without HEMAP in the different food simulants (µg/6 dm^2) is given in Table 1.

Table 1. Specific migration into food simulants

	10% Ethanol (µg/6 dm^2)	3% Acetic acid (µg/6 dm^2)	95% Ethanol (µg/6 dm^2)
Commercial product blank	<4.0	<3.2	<2.76
Commercial product + HEMAP	4.20	24.8	2.81

Overall migration

28. Overall migration was determined with 10% ethanol, 3% acetic acid and olive oil as food simulants for 10 days at 40°C.

29. The original test report noted that a substantial amount of olive oil was absorbed by the samples and as such it was not considered to be suitable for the determination of the overall migration. Therefore overall migration was also determined with 50% ethanol and isooctane for 10 days at 40°C and 95% ethanol for 2 days at 20°C as outlined in assimilated Regulation EU No. 10/2011.

30. All experiments were carried out in triplicate with migration cells to obtain single side contact, with the exception of isooctane and olive oil where total immersion was performed.

31. Table 2 provides the results for overall migration testing. The resulting overall migration for all non-volatile substances was below the overall migration limit of 10 mg/dm^2.

Table 2: Overall migration into food simulants

	10% Ethanol (mg/dm^2)	3% Acetic (mg/dm^2)	50% Ethanol (mg/dm^2)	95% Ethanol (mg/dm^2)	Isooctane (mg/dm^2)
Commercial mixture + HEMAP	0.9 ± 0.2	2.9 ± 0.4	1.3 ± 0.2	0.3 ± 0.0	0.6 ± 0.4

Oligomers and reaction products

32. Commercial samples with and without HEMAP were screened for the presence of volatile, semi-volatile and non-volatile oligomers and reaction products.

33. The migration results for volatiles determined by solid-phase microextraction headspace GC - MS showed one peak in the chromatogram of the sample containing HEMAP, which was not seen in control samples. The peak was tentatively identified as one of the starting substances, given that it was present only in the test sample containing HEMAP and was present in HEMAP itself.

34. The semi-volatile and non-volatile reaction and breakdown products were determined by GC-MS and LC-MS in 3% acetic acid and 10% ethanol following exposure for 10 days at 40°C and 95% ethanol following exposure for 2 days at 20°C.

35. No non-volatile reaction and breakdown products related to HEMAP were detected in the food simulants above the limit of detection of 2 µg/kg.

36. For semi-volatiles (non-polar and polar) the total ion current (TIC) chromatograms in each simulant obtained for the blank (simulant) and samples without HEMAP were compared to the TIC from samples manufactured with the mixture containing HEMAP. All peaks detected in the chromatograms from the food simulants exposed to the test samples with HEMAP were also present in the samples without HEMAP. Hence, no substances were detected that could be related to use of 2-hydroxyethyl methacrylate in the countertop test samples.

37. In 10% ethanol, one substance containing propanoic acid was also tentatively identified, together with other peaks with lower intensities.

38. However, no additional peaks were observed for the samples with HEMAP when compared to the sample without HEMAP, nor in any of the blank food simulant samples.

39. Base-peak chromatograms are not specific enough to detect peaks with low intensities. Therefore, extracted ion chromatograms of the exact masses calculated for the theoretical oligomers and reaction products were used to screen for their presence.

40. Several peaks were present, with some being identified as the main components of HEMAP.

41. The FCMJEG concluded that under the conditions of the manufacture process of the solid surface countertop, the starting substances have little reactivity except towards free radical addition to the growing polymer chain.

Data on residual content of substance in the FCM

42. HEMAP is intended to be used as a minor monomer (0.35%) in the manufacture of acrylic countertops and sinks and is intended to react into the polymer backbone. The di- and tri-substituted phosphoric acid components of HEMAP have multiple reactive functional groups making it therefore less likely to remain unreacted. Therefore, the residual content was not determined but instead, specific migration was determined.

43. The FCMJEG agreed with the Applicant’s conclusion that as the specific migration was low, essentially all of the HEMAP was incorporated into the backbone.

Toxicological data

44. As HEMAP is never separated from the final commercial product, the toxicological data presented below are for the final product and not HEMAP itself.

Genotoxicity

Bacterial reverse mutation test (Ames Test)

45. The study was conducted in accordance with test guideline OECD No. 471, using the plate incorporation method.

46. *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* strain WP2 uvrA were exposed to the test substance with HEMAP at concentrations ranging from 47 – 5000 µg/plate with or without S9-mix (post-mitochondrial supernatant from liver of rats treated with an enzyme inducer) for metabolic activation.

47. Clear and colourless stock solutions of the test substance (+ HEMAP) were prepared at concentrations of 50 mg/mL and 30 mg/mL in dimethyl sulfoxide (DMSO) for Experiments 1 and 2. In all tests performed, the mean numbers of His+ and trp+ revertant colonies, as appropriate, of the negative controls were within the acceptable range and the positive controls gave the expected increase. Therefore, the experiments were considered valid.

48. In Experiment 1, toxicity was observed at concentrations ≥ 1667 µg/plate in all *Salmonella* strains in the absence of S9-mix. A 1.9-fold increase in revertant colonies was noted with *S. typhimurium* TA1537 at 185 µg/plate in the absence of S9-mix. To determine whether this result was biologically relevant, a repeat assay (Experiment 2) was performed with *S. typhimurium* TA1537 without S9 at concentrations of 0, 47, 94, 188, 375, 750, 1500, or 3000 µg/plate. The highest concentration tested in this repeat assay was limited by toxicity observed in the initial assay. In the repeat assay, cytotoxicity was noted at ≥ 750 µg/plate.

49. The test substance (+ HEMAP) induced less than a 2-fold or dose-related increase in revertant colonies compared with that of the solvent control; because the increase noted in the initial assay was not reproducible, it was not considered biologically relevant.

50. Under the conditions of this study, the final product, containing HEMAP was negative/not mutagenic in the bacterial reverse mutation test (Ames test).

In vitro mammalian micronucleus test

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

52. Cultured human lymphocytes were exposed *in vitro* to the test substance (+ HEMAP) in the presence or absence of S9-mix for metabolic activation. Negative and positive controls were run concurrently. DMSO was chosen as the solvent, as the culture medium was deemed unsuitable, due to the formation of fine oil droplets.

53. In Experiment 1, cells were exposed to the test substance for 4 hours, followed by a 20-hour recovery period, at concentrations of 15.6, 31.3, 62.5, 125, 250, 500, 750, 1000, 1500 and 2000 µg/mL with and without S9-mix.

54. In Experiments 2 and 3, cells were exposed to the test substance for 20 hours with no recovery period at concentrations of 15.6 - 2000 µg/mL (Experiment 2, same as in Experiment 1).

55. In experiment 3, cells were exposed to the test substance for 20 hours with no recovery period at concentrations of 50, 100, 200, 300, 375, 425, 500, 600 700 and 800 µg/mL, without S9-mix.

56. The number of binucleated cells containing micronuclei in negative/solvent controls was within the acceptable range and treatment with positive controls resulted in statistically significant increases in the number of such cells, when compared to controls. Therefore, the experiments were considered valid.

57. In Experiment 1, the test substance showed concentration-dependent cytotoxicity, with severe cytotoxicity (72%) at the highest concentration tested (2000 µg/mL) in the presence of S9-mix. Concentrations of 250, 500, 1000, and 1500 µg/mL (selected for micronucleus analysis) resulted in cytotoxicity of 4%, 0%, 17%, and 40%, respectively. The applicant noted that this condition did not meet the aimed cytotoxicity range of the OECD guideline (55 ± 5%). CBP (cytokinesis block proliferation) analysis showed a low cell density on the slides, which indicated a reduction in cell number as a result of the treatment. Therefore, a top concentration with a slightly lower cytotoxicity was selected. The lower selected concentrations (500 and 250 µg/mL) showed no or low cytotoxicity, respectively, when compared to the solvent control. At the concentration of 1000 µg/mL a single culture was used for analysis, since the other culture was considered to be an outlier based on the cytokinesis-block proliferation index (CBPI) (use of single culture is in alignment with the OECD guideline). In the absence of S9-mix, severe cytotoxicity was noted at the two highest concentrations tested (1500 and 2000 µg/mL). Concentrations of 250, 500, and 1000 µg/mL (selected for micronucleus analysis) showed cytotoxicity of 3%, 23%, and 53%, respectively, compared to the control.

58. In Experiment 2, severe cytotoxicity was noted at the four highest concentrations tested (750, 1000, 1500 and 2000 µg/mL) in the absence of S9-mix. The next lower concentration (500 µg/mL) showed 64% cytotoxicity, all other concentrations (250 to 15.6 µg/mL) exhibited little to no cytotoxicity. The applicant noted that 64% cytotoxicity was above the aimed cytotoxicity stated in the OECD guideline. In addition, due to a steep concentration response curve for the test substance, a concentration representing moderate cytotoxicity was not obtained. Although this experiment did not fully meet the target cytotoxicity range of the OECD guideline, three concentrations (125, 250, and 500 µg/mL) together with the solvent control and positive control were selected for analysis of micronuclei induction.

59. In Experiment 3, the test substance showed concentration related cytotoxicity, in the absence of S9-mix. Strong cytotoxicity was noted at the four highest concentrations tested (500, 600, 700 and 800 µg/mL). Concentrations of 100, 300, and 425 µg/mL (selected for micronucleus analysis) showed cytotoxicity of 12%, 29%, and 57%, respectively, compared to the control.

60. There was no statistically significant ($p > 0.05$, Chi-Square one-sided test) increase in the incidence of micronuclei-containing binucleated cells in any experiment at any concentration analysed when compared to concurrent controls. There was no concentration-related increase when assessed for statistical trend.

61. Under the conditions of this *in vitro* micronucleus test, the applicant considered the test substance (+ HEMAP) to be neither clastogenic nor aneugenic by the applicant.

Conclusion by the FCMJEG

62. HEMAP is intended to be used as part of a commercial mixture for use in the production of countertops and sinks.

63. The FCMJEG considered the information on the identity of the substance, the physical and chemical properties and intended application satisfactory. Results from the overall and specific migration tests demonstrated the migration was below the overall migration limit and the specific migration of HEMAP was considered to be acceptable, with the highest specific migration of HEMAP being 24.8 µg/6 dm^2 in 3% acetic acid.

64. The specific migration of the sum of HEMAP plus its phosphate and diphosphate esters under the worst foreseeable conditions of use was 24.8 µg/6 dm^2. It is assumed that this is equivalent to contact with 1 kg food.

65. The toxicological information that formed the basis of the risk assessment was a bacterial reverse mutation test (Ames test), which was conducted in accordance with OECD No. 471, and an in vitro mammalian micronucleus test in accordance with OECD No. 487, on the commercial product. Results of these tests showed no mutagenic, clastogenic or aneugenic potential for the commercial product under the experimental conditions described. Hence, the commercial mixture, containing HEMAP, did not raise any concerns for mutagenic, clastogenic or aneugenic potential, from the results of the genotoxicity tests.

66. Taking into account that the specific migration of the sum of HEMAP plus its phosphate and diphosphate esters is not expected to exceed 50 µg/kg food and the negative results in the Ames and in vitro micronucleus tests, the FCMJEG proposed a specific migration limit (SML) of 0.05 mg/kg food for HEMAP.

67. Overall, the FCMJEG did not see a concern to human health from the use of HEMAP in the commercial product to be used in the manufacture of kitchen countertops and sinks up to a maximum percentage in formulation of 0.35%.