MUTAGENICITY POTENTIAL OF A MODIFIED CLAY PRESENT IN A NANOCOMPOSITE MATERIAL INTENDED TO FOOD PACKAGING AND ITS MIGRATION EXTRACT

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Abstract: Modified clays with ammonium quaternarium salts are currently used to develop new packaging materials for the food industry. These improved materials are known as nanocomposites. In previous works a modified montmorillonite clay, Clay1, and the migration extract from a polylactic acid-Clay1 nanocomposite (PLA-Clay1) have been studied in regard to their possible toxicity to the consumers, due to their potential migration from the nanocomposite to the food [1,2]. But there is no information about their mutagenic potential. The incorporation version of the Ames test was performed according to the recommendations of Maron and Ames [3] and following the principles of the OCDE guideline 471 [4]. Five *Salmonella typhimurium* histidine-auxotrophic strains TA97A, TA98, TA100, TA102 and TA104 were used for the assay. Five different concentrations of Clay1 (0.5-8 µg/mL) and PLA-Clay1 extract (20-100%) were assessed in three independent experiments. Each assay was conducted in absence and presence of S9 metabolic activation system from rats livers, using triplicate plates for each test substance concentration. Revertant colonies were counted and background lawn was inspected for signs of toxicity or compound precipitation. 2-Nitrofluorene (2-NF) (0.1 µg/plate) and sodium azide (NaN_3) (1µg/plate), were selected as positive controls in absence of S9 metabolic fraction and 2-Aminofluorene (2-AF) (20 µg/plate) in presence of S9 metabolic fraction. In general, Clay1 demonstrated a mutagenic effect while PLA-Clay1 extract did not showed any mutagenic effect at the concentrations tested (Table 1). Further safety studies should be carried out before these kinds of materials could be widely used in the food industry.

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References

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Table 1

a	C (µg/mL)	TA97A		TA98		TA100		TA102		TA104	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Clay1	Negative controls	216±28	206±12	21±3	38±14	85±16	80±18	183±25	216±45	252±45	285±45
	0.5	241±75	213±30	25±7	68±12*	91±26	87±17	142±45	166±12	221±51	228±54
	1	236±69	254±55	29±10	144±22#	79±15	79±24	180±25	230±43	252±69	236±54
	2	211±54	254±51	35±10	303±100#	70±11	88±18	189±48	173±66	270±73	367±69
	4	262±70	260±87	28±8	521±26#	89±21	85±18	281±72	242±67	309±51	341±86
	8	211±26	260±51	34±20	150±61#	80±15	89±32	218±84	202±41	295±47	323±83
	Positive controls	387±60#	485±86#	>1000#	>1000#	347±50#	411±120#	483±51#	541±29#	749±120#	>1000#

b	C (%)	TA97A		TA98		TA100		TA102		TA104	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
PLA-Clay 1 Extract	Negative controls	338±54	402±18	21±4	20±1	115±25	123±34	246±40	246±65	320±59	326±26
	20%	368±2	286±67	23±9	22±6	100±26	104±35	221±92	236±30	349±7	320±99
	40%	409±65	438±10	21±3	24±5	109±19	101±40	324±76	352±62	409±65	418±40
	60%	320±11	424±11	27±4	32±2	124±20	122±1	353±120	365±57	307±16	284±22
	80%	343±42	357±22	18±1	25±1	107±29	96±36	256±9	297±47	295±61	256±42
	100%	271±49	342±34	21±3	30±5	154±8	143±1	272±53	260±8	266±41	286±40
	Positive controls	751±24#	722±28#	>1000#	>1000#	>1000#	>1000#	621±172#	853±174#	884±79#	>1000#

Table 1. Results of the Ames test conducted with Clay1 (a) and PLA-Clay1 extract (b) in three independent experiments. Water was used as negative control and DMSO as solvent for positive controls. Data are given as mean±SD revertants/plate for three replicates for each concentration in each experiment. Positive controls: TA97A/ TA98/ TA102/ TA104 without S9: 2-NF (0.1 µg/plate) and TA100 without S9: NaN_3 (1µg/plate). 2-AF was used in presence of S9. *p<0.05 and #p< 0.01 significant and very significant different from control, respectively.