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ORIGINAL ARTICLE

Genotoxicity Assessment of Ultra-High Molecular Weight Polyethylene (UHMWPE) using Bacterial Reverse Mutation and In vitro Chromosomal Aberration Assays

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Abstract

Objectives: Ultra High Molecular Weight Polyethylene (UHMWPE) is extensively used in orthopedic and implantable medical devices, yet data on its genotoxic potential remain limited. This study aimed to comprehensively assess the mutagenic, clastogenic, and aneugenic potential of UHMWPE extracts using two standard in vitro assays. **Methods:** Following ISO 10993 and OECD guidelines (471 and 473), UHMWPE extracts were prepared using polar and non-polar solvents. Mutagenicity was evaluated using Salmonella typhimurium strains (TA98, TA100, TA102, TA1535, and TA1537) with and without S9 metabolic activation. *In vitro* Chromosomal aberration analysis was performed on cultured human peripheral blood lymphocytes exposed to UHMWPE extracts at 25%, 50%, and 100% concentrations under both short-term (±S9) and long-term (-S9) exposure conditions. **Findings:** In the Ames test, no significant increase in revertant colonies was observed across strains, indicating no mutagenic activity. Revertant counts for the test material ranged from 125 ± 6 to 130 ± 6 CFU/plate, similar to negative controls (e.g., TA100: 126 ± 6 vs. 136 ± 13). In contrast, positive controls like mitomycin C showed marked increases (e.g., TA102: 703 ± 35; TA1537: 351 ± 15), confirming assay validity. In the chromosomal aberration assay, the test material induced no significant structural or numerical aberrations, with aberrant cells at 1%–4% (vs. 14%–22% for positives). The mitotic index remained within normal range (8.5 with S9; 8.8 and 7.6 without S9), and numerical aberrations stayed below 1%, unlike positive controls (up to 5.7%). **Novelty:** This study uniquely evaluated UHMWPE extractables (not wear particles) for genotoxicity using both assays under internationally accepted regulatory protocols. It provides new safety evidence supporting the non – mutagenic and non – clastogenic nature of UHMWPE in medical device applications.

Keywords: UHMWPE; Genotoxicity; Ames Test; Chromosomal Aberration; Medical Devices; Biocompatibility

1 Introduction

In modern healthcare, medical devices have become essential for diagnosing, monitoring, and treating various medical conditions. Biocompatibility, which ensures the materials used are compatible with biological systems, is critical in developing these devices.

To assess the risk of medical devices, it is vital to use non-toxic or low-toxicity materials and follow the ISO 10993 guidelines, including *in vitro* and *in vivo* tests. *in vivo* assays help detect hazards such as genotoxicity and irritation and aid in material selection. *in vivo* tests, despite species differences, provide additional data that complements *in vitro* results. Together, these tests evaluate potential toxicity and overall safety.[(1)]

UHMWPE is an ethylene homopolymer with a highly cross-linked, crystalline structure (>90% crystallinity), offering excellent biocompatibility, chemical inertness, and high tensile strength.

UHMWPE is widely used in orthopedic implants due to its excellent wear resistance and mechanical performance. Despite its popularity, concerns remain about potential adverse biological effects, especially related to its leachates and degradation products.[(2)]

The Ames Mutagenicity test determines whether leachable from a medical device or material could cause mutations. This assay assesses the potential for mutagenic activity in extracts from the device of material.[(3)]

The *in vitro* chromosomal aberration test aims to detect agents that induce structural chromosomal abnormalities in cultured mammalian cells. An increase in polyploidy may suggest a chemical's potential to cause numerical chromosomal aberrations.[(4)]

The Chromosomal Aberration Assay plays a vital role in assessing the genotoxic potential of medical devices, ensuring their safety at the chromosomal level. As a key component of the safety evaluation process, this assay directly impacts patient well-being by confirming that the device does not induce genotoxic harm. This proactive approach helps manufacturers identify and mitigate genotoxic risks early in the device development process, well before commercialization.[(5)]

Although UHMWPE has been extensively studied for mechanical strength, wear resistance and clinical, performance[(6)],[(7)],[(8)], there is limited research evaluating its genotoxic potential from chemical extractables.

Limited research exists on the potential genotoxic effects of extractables and leachables from UHMWPE itself. Most investigations have focused on particle-mediated responses rather than the chemical safety of leachable compounds. Studies on antioxidant-stabilized UHMWPE and recycled plastics[(9)],[(10)] have shown chemical extractables may exist, but their mutagenicity has not been systematically tested. This study fills that gap by evaluating the genotoxic potential of UHMWPE extract using both Ames and chromosomal aberration assays in compliance with ISO 10993-3 and OECD 471/473 guidelines.

This study focuses on evaluating the genotoxic effects of UHMWPE extracts using the bacterial reverse mutation (AMES) test and in vitro chromosomal aberration assay. These tests are selected based on the international guidelines (ISO 10993-3, ISO/TR 10993-33 and OECD 471/473), ensuring a standardized and relevant safety assessment. While many studies have explored cytotoxicity and inflammatory responses from UHMWPE particles, fewer have examined its intrinsic genotoxic potential. Regulatory bodies like ISO and OECD emphasize the need to assess genotoxicity for materials in prolonged or permanent contact with the body.

By investigating the extractable components of UHMWPE rather than the physical wear particles, this research provides a direct understanding of the biocompatibility and material's chemical safety profile, an area less addressed in previous literature. The findings help fill an important gap in the biological evaluation of UHMWPE for long term implant use.

2 Methodology

The genotoxic evaluation of UHMWPE was conducted following ISO 10993 – 12 for sample preparation and ISO 10993 -3, 33 for biological testing, alongside OECD guidelines 471 and 473.

2.1 Test Material

UHMWPE (supplier details were not available but the material met the medical-grade quality) component of hip implants and an irregularly shaped solid device, was evaluated in this study using a standardized extraction ratio of 0.2 g/mL, following ISO 10993-12 guidelines.

2.2 Preparation of Extracts

The extraction conditions used for the bacterial reverse mutation (Ames) Assay and chromosomal aberration assay in compliance with ISO 10993-12.[(11)]

The test material 3.45g in 17.3 ml and 3.48g in 17.4 was extracted using physiological saline (polar) and DMSO (non polar) for the Ames and RPMI medium for the chromosomal aberration assay. Dimethyl sulfoxide (DMSO) was selected as the organic extraction vehicle recommended by the standard for assessing potential non-polar and semi-polar constituents. Extraction of the test material was performed in accordance with ISO 10993-12:2021 guidelines. Extraction was performed at 50°C (polar/non polar) and 37°C (RPMI) for 72 hrs. Extracts were clear, no color change and no presence of particulates were. No additional processing such as filtration, centrifugation, pH adjustments were made. The prepared extract was then used for genotoxicity testing.

2.3 Bacterial Reverse Mutation (AMES) Assay

2.3.1. Chemicals and reagent preparation

The concentrations of controls, along with the preparation details of chemicals and media, are summarized in the Table 1, 2 below. The use of metabolic activation (S9 and non-S9) with rat liver enzymes is also indicated in relation to the positive controls for each bacterial strain.

Table 1. Controls and Concentration used

Negative control	Positive Control	Concentration (mL per plate)
Physiological Saline (Polar Solvent)	Benzopyrene	0.1
Dimethyl sulphoxide (DMSO - Non-Polar Solvent)	Sodium Azide	0.1
NA	2-Nitrofluorene	0.1
NA	9-Aminoacridine	0.1
NA	Mitomycin - C	0.1

Table 2. Preparation of chemicals and medium

S. No	Chemical Name	Quantity used (μg/mg/ml)	Preparation method
1	Vogel-Bonner Minimal Medium E		
	Magnesium sulfate	0.8 g	
	citric acid monohydrate	8 g	Dissolved in 48 ml of water stirred using magnetic stirrer for 15 mins
	Potassium phosphate	40 g	
	Sodium ammonium phosphate tetrahydrate	14 g	
2	40% Glucose Solution	80 g	Dissolved in 120 ml of distilled water
3	Oxoid Nutrient Broth	1.5 g	Dissolved in 60 ml of distilled water
4	Basal Agar	51 g	3162 ml of distilled water
5	Magnesium chloride	5.08 g	15 ml of distilled water
6	Histidine - Biotin Solution		
	Histidine	20 mg	Dissolved in 20 ml of 250 mM magnesium chloride solution and 20 ml of distilled water
	Biotin	20 mg	
7	Overlay (Top) Agar		
	Bacto agar	0.5%	Dissolved in 350 ml of double distilled water
	NaCl	0.5%	
8	Metabolic activation system (+S9 mix)		
	10% pre-mixed mutazyme with rat liver S9		
	10% pre-mixed mutazyme with liver S9		
	treated with sodium phosphate buffer solution		
9	Minimal Glucose agar		
	Bacto agar	51 g	Dissolved in 3162 ml of distilled water VB salts and glucose solutions were added and ingredients are mixed and poured in petri dishes
	50X Vogel-Bonner (VB) salts (68 mL)	68 ml	
	Sterile 40% glucose solution	170 ml	

All the chemicals and reagents were purchased from Sigma Aldrich. Refer Table 3 for the Metabolic Activation system.

Table 3. Strains & Positive controls +/- S9 metabolic activation

Salmonella typhimurium Strains	Positive Controls	
	With S9 Metabolic Activation (conc .μg/plate)	**Without S9 Metabolic Activation (conc. μg/plate)**
TA 100, TA 1535	Benzopyrene (5,10)	Sodium Azide
TA 98	Benzopyrene (5)	2-Nitrofluorene
TA 1537	Benzopyrene (10)	9-Aminoacridine
TA 102	Benzopyrene (10)	Mitomycin - C

Test System:

The strains TA 98, TA 100, TA 1535, TA 1537, TA 102 of *Salmonella typhimurium* were procured from Molecular Toxicology Inc., USA. Oxoid nutrient broth was served as growth medium, and the strains were maintained at 37 $\pm^{\circ}$C for 10h in a shaker water bath at120 rpm with $\sim 10^9$ cells/culture.

A small portion of Salmonella typhimurium strains (TA98, TA100, TA102, TA1535, TA1537) was scooped out using an ice-cold spatula and inoculated into respective flasks in Biosafety Cabinet Class II (BSC II) under aseptic conditions. The flasks were placed in a shaker water bath, and the temperature was set to 37 $\pm$1 $^{\circ}$ C and agitated at 120 rpm for 10 h. After incubation, the shaker water bath was programmed to maintain the cultures at 4 $^{\circ}$ C until use.

Table 4. Strains and Type of Mutagenicity

Strains	Mutagenicity
TA 98	Frame shift
TA 1535	Base pair substitution
TA 100	Base pair substitution
TA 1537	Frame shift
TA 102	Base pair substitution

The bacterial strains were selected in accordance with OECD TG 471 and ISO 10993-3 guidelines to cover a broad spectrum of mutagenic events. Specifically, TA98 and TA100 detect frameshift and base-pair substitution mutations, respectively, while TA102 is sensitive to oxidative mutagens and DNA-damaging agents. This selection ensures that the assay addresses the mutagenic potential of the test material comprehensively, as recommended by the guidelines.

2.3.2. In vitro Chromosomal Aberration Assay

Chemicals and reagents preparation:

Positive Control

Mitomycin-C (MMC) and Cyclophosphamide (CPA) were obtained from Sigma respectively and are used as positive controls respectively. MMC was dissolved in distilled water and used at concentrations of 0.40 and 0.80 μg/mL for treatments in the absence of S-9. CPA was also dissolved in distilled water and used at concentrations of 6.25 and 12.5 μg/mL for treatments in the presence of S-9. Untreated cultures served as solvent control.

Test System:

The study was designed in accordance with ISO 10993-3:2014(E) and OECD Guideline No. 473 (adopted on July 29, 2016) for the assessment of chromosomal aberrations.

Peripheral blood samples (11 mL per donor) were collected from three healthy, non-smoking male volunteers with no recent history of illness or medication. The use of human blood samples in this study was approved by the Institutional Ethics Committee, and informed consent was obtained from all donors. The samples were pooled in equal quantities for culture preparation [12]. Whole blood cultures were established using RPMI minimal medium, fetal bovine serum, L-glutamine, penicillin/streptomycin, and phytohemagglutinin (PHA-M). The cultures were maintained under standard conditions with 8% pooled blood. The metabolic activation system consisted of a 10% mammalian liver post-mitochondrial fraction, stored at -20°C and thawed prior to use. A 0.5 mL aliquot of mutazyme (5% S-9 mix) was added to cultures treated in the presence of S-9, while an equivalent volume of 150 mM KCl was added to cultures treated in the absence of S-9. Each culture tube was labeled with the study number, S-9 mixture details, treatment time, test material concentration, and control identifiers (positive, solvent, or untreated controls). All chemicals and reagents used in this study were obtained from Sigma Aldrich.

2.4 Experimental Procedure

2.4.1. Bacterial Reverse Mutation (Ames) Assay

The mutagenic potential of the test extracts was evaluated using the plate incorporation method with five Salmonella typhimurium strains (TA98, TA100, TA102, TA1535, and TA1537). The extracts were tested at 100% concentration in both polar and non-polar solvents, with and without the S-9 metabolic activation system. Triplicate plates were used for each test condition, including positive and negative controls in the presence and absence of the S-9 mix. (10) The mixture of 0.1 mL of freshly grown bacterial culture, 0.1 mL of the test material extract or control, 0.5 mL of either 10% S-9 mix, or sodium phosphate buffer solution was rapidly mixed and immediately poured onto Vogel-Bonner E agar plates. After the agar was set, the plates were inverted and incubated at 37°C in the dark for 48 hours. Following incubation, plates were examined for signs of toxicity and the revertant colonies are counted manually. The experiment was performed in triplicates, and a summary of data is presented. The mean number of revertant colonies per plate and the standard deviation (SD) were calculated for the test material, as well as for positive and negative controls.

2.4.2. In vitro Chromosomal Aberration Assay

Whole blood cultures were treated with the test material extract under three exposure conditions of 3+17 hours with and without S-9 mix and 20+0 hours without S-9 mix. CPA served as the positive control at concentrations of 6.25 and 12.5 μg/mL for of 3+17 hours with S-9 mix. MMC served as positive control at concentrations of 0.40 and 0.80 μg/mL for 3+17 hours and 20+0 hours without S-9 mix.

Approximately 48 hours after culture initiation, 1.0 mL of test material stock solution and 0.5 mL of S-9 mix or 150 mM KCl were added. The final culture volume was adjusted to 10 mL. Cultures were incubated at 37°C for 3 or 20 hours, depending on the treatment regimen. Following the 3-hour treatment, cells were centrifuged, washed twice with saline, and resuspended in fresh medium for an additional 17-hour incubation. Three hours prior to harvest, 0.1 mL of colchicine (0.1 mg/mL) was added to arrest cells in metaphase. At the end of the treatment period, the pH of the culture medium was checked, and no significant changes were observed. Cells were centrifuged and subjected to hypotonic treatment with 5ml of 0.075 M KCl at 37°C for 15 minutes, followed by fixation in methanol: acetic acid (3:1, v/v) overnight at 4°C. Fixed cells were washed, resuspended, and slides were prepared. Two slides per culture were prepared by placing a few drops of fixed cell suspension onto clean, labeled glass slides and drying them on a slide warmer. The slides were stained with Giemsa stain for 10 minutes and subsequently analyzed.

A total of 1000 cells per culture were scored to determine the mitotic index (MI) and mitotic inhibition percentage (MIH%).

Mitotic Index (MI) was calculated as:

$$\text{MI} = \frac{\text{Number of cells in mitosis}}{\text{total number of cells observed}} \times 100$$

Mitotic Inhibition (MIH%) was calculated as:

$$\text{Mitotic inhibition (MIH \%)} = [1 - (\text{mean MI}_\text{T}/\text{mean MI}_\text{C})] \times 100\%$$

$$(\text{Where T} = \text{treatment and C} = \text{negative control})$$

Mitotic index was used as an indicator of cytotoxicity. Since the neat extract did not induce cytotoxicity, all tested concentrations (100%, 50%, and 25%) were analyzed for chromosomal aberrations. Two slides per culture from all test concentrations were analyzed microscopically. Positive control slides (MMC and CPA) were examined to confirm the validity of the test system.

A total of 200 metaphases per concentration were analyzed for structural and numerical aberrations, with only cells containing 44-48 chromosomes considered for evaluation. Polyploid, hyperdiploid, or endoreduplicated cells were recorded separately. Gaps, defined as chromatid discontinuities without displacement, and deletions, defined as discontinuities with fragment displacement, were recorded accordingly.

3 Results and Discussion

The safety assessment of test material UHMWPE has been investigated but the genotoxicity has not been verified. The biocompatibility of UHMWPE was assessed through the in vitro genotoxicity test.

3.1 Bacterial Reverse Mutation (Ames) Assay

As shown in Table 5, the negative and positive controls were performed as expected. The values were consistent with historical control data, and the positive controls confirmed the validity of the assay, indicating the absence of mutagenic potential under the test conditions. In the present study, UHMWPE did not include significant increases in the number of revertant colonies across all Salmonella typhimurium strains, either with or without metabolic activation, when compared to negative controls. No thinning of the background lawn was observed in any of the extract treated plates. The mean number of revertant colonies obtained with all implant extracts indicates no mutagenicity of bacterial reverse mutation test with or without S9 mixtures under the experimental conditions.

Table 5. Revertant mutant colonies

Extracts	Volume / Concentration	S9 mix	Average Histidine Revertant colonies / Plate (CFU/Plate)															
			TA100			TA102			TA 1535			TA1537			TA98				
NC - Phy.sal	100 μL	X	136	±	13	250	±	11	20	±	2	21	±	5	27	±	4	
NC - Phy.sal		✓	125	±	5	239	±	29	7	±	3	18	±	7	28	±	4	
NC-DMSO	100 μL	X	137	±	13	254	±	18	15	±	6	20	±	6	31	±	5	
NC-DMSO		✓	126	±	6	247	±	16	10	±	3	22	±	2	29	±	2	
Positive Control	0.5 μg	X		NA		703	±	35		NA			NA			NA		
Mitomycin C	1 μg	X	641	±	28		NA		320	±	53		NA			NA		
Sodium azide	10 μg	X		NA			NA			NA			NA		250	±	50	
2 - Nitrofluorene	50 μg	X		NA			NA			NA		351	±	15		NA		
9 - Aminoacridine	5 μg	✓	661	±	37		NA			NA			NA		270	±	36	
Benzo (a) pyrene	10 μg	✓		NA		690	±	15	388	±	34	250		92		NA		
Phy.Sal	100 μL	X	147	±	29	254	±	16	13	±	2	20	±	2	29	±	6	
Test material in Phy.Sal	(100% extract)	✓	126	±	6	250	±	25	7	±	2	20	±	1	30	±	6	
DMSO	100 μL	X	134	±	3	258	±	29	10	±	12	22	±	4	30	±	4	
Test material in DMSO	(100% extract)	✓	125	±	6	236	±	8	19	±	9	20	±	2	28	±	2	

Values are Mean ± SD of 3 plates; X - without S9 mix, ✓ - with S9 mix; NA - Not Applicable, NC -Phy.Sal – Negative control Physiological saline, NC-DMSO – Negative control Dimethyl sulphoxide, Phy Sal-Physiological Saline, DMSO - Dimethyl sulphoxide

3.2 In Vitro Chromosomal Aberration Assay

The cytotoxicity assessment, for the test material extract (25%, 50%, 100%) was determined based on the maximum cytotoxicity concentrations obtained 17.5, 6.4 and 4.6% for 3+17 hours with and without S-9 mix and 20+0 hours without S-9 mix and these concentrations were selected for chromosomal aberrations analysis. At each selected concentration, a total of 200 metaphase cells per culture were analyzed for chromosomal aberrations. The chromosomal aberration frequencies in solvent controls were within the negative control ranges. The positive control groups exhibited a significant increase in chromosomal aberrations, confirming the validity of the assay.

Structural chromosomal aberrations were evaluated at different treatment durations: 3+17 hours with and without S-9 and 20+0 hours S-9. The results indicated that the solvent control showed minimal aberrations, which were within the historical negative control range, confirming assay reliability.

For the test material, structural aberrations were observed at 100% concentration, with a slight increase in the number of aberrant cells compared to the solvent control. However, the frequency of aberrations was significantly lower than the positive controls, indicating a lack of strong genotoxic potential. The highest concentration (100%) exhibited a total of 7 aberrant cells (3+17h, +S9), 5 aberrant cells (3 +17h, -S9) and 2 aberrant cells (20+0h, -S9), excluding gaps. The analysis of no. and types of structural aberrations were given in Table 6.

Numerical chromosomal aberrations were also assessed, and the solvent control exhibited low frequencies, consistent with historical data. The test material extract at all concentrations showed numerical aberrations at frequencies comparable to the

solvent control, with the highest observed at 100% concentration, where frequency ranged from 0.7% to 1.3% across conditions. This indicates no significant induction of numerical aberrations by the test material.

The mitotic index showed a concentration-dependent decrease, with a notable reduction at 100%, suggesting mild cytotoxicity at the highest test concentration. However, the mitotic index values remained above the threshold indicative of excessive toxicity, ensuring valid assay conditions.

The positive controls induced a significant increase in both structural and numerical aberrations, demonstrating the assay's sensitivity in detecting clastogenic and aneugenic effects. The numbers and types of numerical aberrations were given in Table 7. The positive and negative control images of chromosomal breakage and chromatid gaps were given in Figure 1.

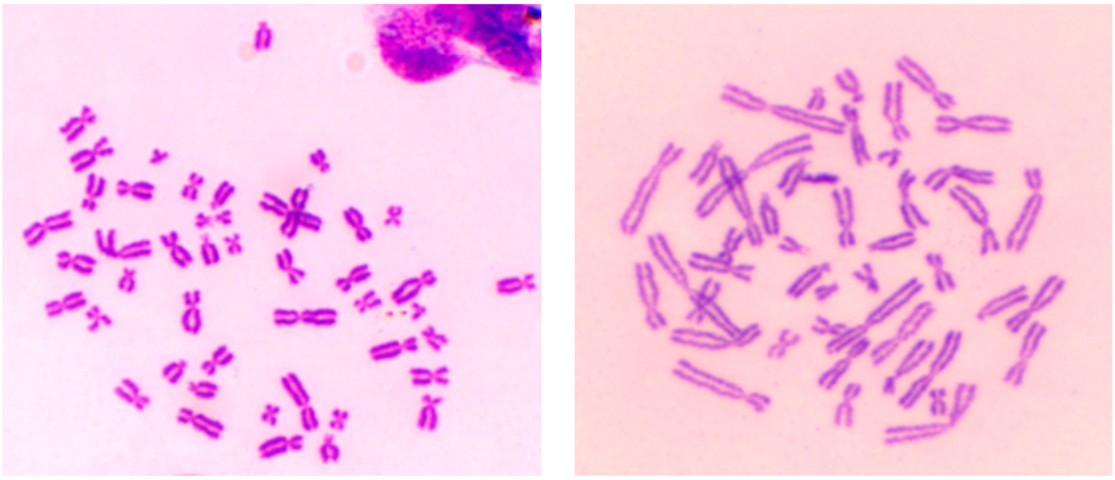

Fig 1. Structural Aberration. A. Negative control - chromosomal break, gap in chromatid, B. Positive control - chromosomal break, gap in chromosome & chromatid

The results were interpreted descriptively by comparing the proportion of aberrant cells in treated cultures with concurrent negative controls and laboratory historical control ranges, as recommended by ISO 10993-3 and OECD TG 473. Because the frequencies of aberrant cells for all test concentrations were within the concurrent and historical control ranges and no concentration-related increase was observed, formal statistical tests (e.g., Chi-square or ANOVA) were not applied. This approach is consistent with guideline recommendations to consider biological relevance before statistical significance.

Ultra-High Molecular Weight Polyethylene (UHMWPE) has gained widespread use in biomedical devices due to its superior wear resistance, chemical stability, and mechanical performance. However, long-term biocompatibility and potential genotoxic effects from extractable substances remain critical for regulatory safety, especially for materials implanted in the human body. According to ISO 10993-3 and 10993-33, genotoxicity testing using both bacterial reverse mutation and chromosomal aberration assays is essential for evaluating genetic safety of such materials.

Recent reviews by Hasegawa and Sudo[6] have detailed advances in wear resistance and oxidative stability of polyethylene in arthroplasty, they do not address the potential genotoxic risks from extractable compounds. Our study bridges this gap by evaluating UHMWPE extractables using standardized mutagenicity and clastogenicity assays, providing crucial biological safety data missing in prior reviews.

While many studies have explored UHMWPE's mechanical and tribological performance, relatively few have addressed its biological risk profile in depth. Wahed et al.[7] reported on cellular responses to UHMWPE, including fibroblast attachment and inflammatory markers, but did not investigate mutagenicity or chromosomal effects. Similarly, Scemama et al.[8] provided long-term clinical insight on vitamin E–blended UHMWPE implants, yet their analysis lacked chemical safety or genotoxic evaluation.

Studies on antioxidant-stabilized UHMWPE (e.g., Vitamin E-stabilized formulations) suggest improved oxidative stability[9], which may indirectly reduce harmful extractables. And miniaturized mutagenicity testing has been applied to plastics[10], none of these approaches included both bacterial reverse mutation and chromosomal aberration assays. Our

Table 6. Number & Types of Structural Aberrations

Treatment condition	Test Material extract concentration (%)	S9 Mix	Total no. of Cells	G	Chr del	Chr exch	Ctd del	Ctd exch	Other	Abs +g	Abs -g	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Mitotic Index (mean)
3 + 17 hour	Solvent	+	400	6	0	0	0	0	0	6	0	7	0	10
	25	+	300	1	0	0	0	0	0	1	0	4	1	10
	50	+	300	3	0	0	1	0	0	4	1	4	1	9.4
	100	+	300	5	0	0	1	0	0	6	0	8	1	8.5
	CPA; 6.25 μg/mL	+	138	6	6	3	7	2	0	24	18	28	20	ND
	CPA; 12.5 μg/mL	+	140	8	2	3	6	3	0	22	15	26	17	ND
3 + 17 hour	Solvent	-	400	3	0	0	0	0	0	3	0	5	2	9.2
	25	-	300	1	0	0	2	0	0	3	2	4	2	9.3
	50	-	300	2	0	0	0	0	0	2	0	3	0	9.2
	100	-	300	1	1	0	2	0	0	4	3	5	4	8.8
	MMC; 0.40 μg/mL	-	138	5	2	3	9	2	0	21	16	23	18	ND
	MMC; 0.80 μg/mL	-	115	4	6	3	9	2	0	23	20	26	22	ND
20 + 0 hour	Solvent	-	400	2	0	0	0	0	0	2	0	4	1	8.5
	25	-	300	2	0	0	0	0	0	2	0	2	0	8.5
	50	-	300	1	0	0	0	0	0	1	0	2	0	8.4
	100	-	300	1	0	0	1	0	0	2	1	3	1	7.6
	MMC; 0.40 μg/mL	-	140	2	2	3	7	2	0	16	14	16	14	ND
	MMC; 0.80 μg/mL	-	129	7	6	3	9	3	0	28	21	30	23	ND

ND – Not determined, G - Gap, Chr del – Chromosome deletion, Chr exch – Chromosome exchange, Ctd del - Chromatid deletion, Ctd exch – Chromatid exchange, Abs +g – Aberrations including gaps, Abs -g – Aberrations excluding gaps.

study strengthens the safety database by confirming that standard-grade UHMWPE does not release mutagenic or clastogenic extractables under simulated physiological conditions.

In contrast, our study focuses specifically on extractable-induced genetic toxicity, which is particularly relevant for systemic or local exposure following long-term implantation. Previous literature, such as Bijukumar et al.[12], identified oxidative stress and DNA damage in macrophages exposed to UHMWPE wear debris. However, their findings were based on particle-induced responses, whereas our work evaluates leachables obtained under controlled extraction conditions as per ISO 10993-12, addressing a separate and equally important toxicological pathway.

Singh and Verma[13] reviewed UHMWPE's use in prosthetics and implants, emphasizing mechanical reliability, yet no experimental data on biological risks were included.

More recent developments have focused on material modifications to improve biological performance. For instance, Kadhim et al.[14] enhanced UHMWPE's cellular compatibility through incorporation of bioactive fillers but did not include genotoxicity assessments. Nancy Hassanein et al.[15] demonstrated improved bioactivity with a novel surface coating; again, their work lacked genetic safety endpoints. Our findings thus add experimental evidence to this body of literature, confirming that unmodified UHMWPE extractables are neither mutagenic nor clastogenic, aligning with international safety requirements.

Tribological studies have also continued to advance. Ortega et al.[16] investigated TiN-coated UHMWPE composites, demonstrating reduced wear but did not explore genotoxic effects. Similarly, Hasan et al.[17] analyzed UHMWPE-HNT composites for joint replacements, reporting mechanical and cytocompatibility results without assessing mutagenicity. Li et al.[18] highlighted current research into high-performance UHMWPE composites and coatings, reflecting a shift towards enhancing functionality. Yet, most studies still overlook comprehensive genotoxic evaluations. These gaps highlight the novelty

Table 7. Numbers and Types of Numerical Aberrations

Treatment condition	Test Material extract concentration (%)	S9 Mix	Total no. of Cells	H	E	P	Hypo	Total abs	% of cells with numerical abs
3 + 17 hour	Solvent	+	400	5	0	0	0	5	1.2
	25	+	300	0	0	0	0	0	0
	50	+	300	3	0	0	0	3	1
	100	+	300	2	0	0	0	2	0.7
	CPA; 6.25 μg/mL	+	138	4	0	0	0	4	2.8
	CPA; 12.5 μg/mL	+	140	7	0	0	0	7	4.8
3 + 17 hour	Solvent	-	400	5	0	0	0	5	1.2
	25	-	300	1	0	0	0	1	0.3
	50	-	300	1	0	0	0	1	0.3
	100	-	300	2	0	0	0	2	0.7
	MMC; 0.40 μg/mL	-	138	6	0	0	0	6	4.2
	MMC; 0.80 μg/mL	-	115	7	0	0	0	7	5.7
20 + 0 hour	Solvent	-	400	3	0	0	0	3	0.8
	25	-	300	1	0	0	0	1	0.3
	50	-	300	2	0	0	0	2	0.7
	100	-	300	3	0	0	0	3	1
	MMC; 0.40 μg/mL	-	140	7	0	0	0	7	5
	MMC; 0.80 μg/mL	-	129	7	0	0	0	7	5.4

CPA – Cyclophosphamide, MMC - Mitomycin-C, H – Hyperdiploid (49-68 chromosomes), P – Polyploid (greater than 68 chromosomes), E – Endoreduplicated, abs – Aberrations

of our study, which combines mutagenicity (Ames test) and clastogenicity (chromosomal aberration assay) to offer a complete genetic safety profile.

We have conducted genotoxicity studies aligned to current regulatory standards, results of genotoxicity study highlight the non-mutagenic and non clastogenic effects of UHMWPE. The data generated from this combined study can be safely used to perform safety assessment of medical devices.

In our Ames test results, the test material extract did not induce mutagenic responses in any of the five *Salmonella typhimurium* strains (TA98, TA100, TA1535, TA1537, and TA102), both in the absence and presence of metabolic activation (S9 mix). The number of revertant colonies remained comparable to the solvent controls, and no dose-dependent increase was observed, thus confirming the absence of base-pair substitutions or frameshift mutations under standard conditions. Positive controls, including sodium azide and 2-nitrofluorene, yielded expected increases in revertants, validating assay responsiveness.

We conducted *in vitro* mammalian chromosomal aberration assay, assessing the structural and numerical chromosomal aberrations using both metabolic activation (+S9) and non-activation (-S9) conditions at multiple exposure durations (3+17 h and 20+0 h). The test material extract was evaluated at increasing concentrations, including up to 100% strength, and results were benchmarked against historical solvent controls and positive controls - Mitomycin C (MMC) and Cyclophosphamide (CPA) - to confirm assay validity.

The solvent controls consistently exhibited minimal chromosomal aberrations, falling within historical negative control ranges, thus confirming the reliability of the assay system. As expected, the positive controls induced significant structural and numerical aberrations under all conditions, validating the assay's sensitivity to both clastogenic (structural) and aneugenic (numerical) agents.

For the test material, a slight increase in structural chromosomal aberrations was observed at the highest concentration (100%), with 7 aberrant cells in 3+17 h (+S9), 5 in 3+17 h (-S9), and 2 in 20+0 h (-S9) treatment groups (excluding gaps). However, these frequencies were substantially lower than those observed in positive controls and did not suggest a strong genotoxic response. Additionally, numerical aberrations at all concentrations remained comparable to the solvent controls, with maximum frequencies ranging between 0.7% and 1.3%, indicating no statistically or biologically significant induction of aneuploidy or polyploidy.

Furthermore, the mitotic index exhibited a concentration-dependent decrease, suggesting mild cytotoxicity at the 100% test concentration. However, all values remained above the cytotoxicity threshold recommended by OECD guidelines and ISO 10993-3, thus ensuring that test conditions did not compromise assay integrity or cell proliferation capacity.

In summary, our findings reinforce that UHMWPE extractables are non-genotoxic, supporting its continued use in implantable medical devices. The dual-approach—using both Ames and chromosomal aberration assays—adds robustness to our conclusions and fulfills a critical component of preclinical risk assessment.

4 Conclusion

UHMWPE is considered non-mutagenic and non-clastogenic under the conditions of this study. These results support its continued use as a biocompatible material for medical devices.

However, it is important to recognize that in vitro assays cannot fully replicate the complexity of in vivo physiological conditions, where factors such as metabolic activation, immune response, and long-term material–tissue interactions may influence genotoxic outcomes. Therefore, future studies should include in vivo genotoxicity testing, such as rodent micronucleus or comet assays, and long-term implantation studies to confirm these findings under physiological conditions. Such data will contribute to a more comprehensive biocompatibility and safety evaluation of UHMWPE used in medical implants.

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6 Contributory statement

Kalaivani. K: Writing – review & editing, Writing – original draft. Dr. S. S. Murugan: Review and editing, Project administration, Supervision. Dr. T. S. Kumaravel: Investigation, Project administration, Review and editing.

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