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Kumaravel, TS

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Tirukalikundram S Kumaravel[a,b,*], Tiruvathipuram Narasimmabarathy Sathya[b], Ramalingam Balaje[b], Pitchaipillai Pradeepa[b], Desikan Yogaraj[b], Malliga Raman Murali[b], Kothamasi Radhakrishnan Navaneethakrishnan[b], Sivasubramanian Murugan[a,b], Awadhesh N Jha[c]

[a] GLR Laboratories (Europe) Pvt. Ltd., Sharnbrook, MK44 1LZ, United Kingdom.

[b] GLR Laboratories Pvt Ltd, Chennai, 600068, India.

[c] School of Biological and Marine Sciences, University of Plymouth, Plymouth, PL4 8AA, United Kingdom.

***Corresponding author:**
Dr. T.S. Kumaravel
GLR Laboratories (Europe) Pvt. Ltd.
The Exchange, Colworth Science Park
Sharnbrook MK44 1LZ
United Kingdom
kumaravelts@glrlabs.com
+44-7767048696

Abstract

This review critically evaluates our current regulatory understanding of genotoxicity testing and risk assessment of medical devices. Genotoxicity risk assessment of these devices begins with the evaluation of materials of construction, manufacturing additives and all residual materials for potential to induce DNA damage. This is followed by extractable and/or leachable (E&L) studies to understand the worst case and/or clinical exposures, coupled with risk assessment of extractables or leachables. The TTC (Threshold of Toxicological Concern) approach is used to define acceptable levels of genotoxic chemicals, when identified. Where appropriate, *in silico* predictions may be used to evaluate the genotoxic potentials of identifiable chemicals with limited toxicological data and above the levels defined by TTC. Devices that could not be supported by E&L studies are evaluated by *in vitro* genotoxicity studies conducted in accordance with ISO10993-3 and 33. Certain endpoints such as 'site of contact genotoxicity' that are specific for certain classes of medical devices are currently not addressed in the current standards. The review also illustrates the potential uses of recent advances to achieve the goal of robust genotoxicity assessment of medical devices which are being increasingly used for health benefits. The review also highlights the gaps for genotoxicity risk assessment of medical devices and suggests possible approaches to address them taking into consideration the recent advances in genotoxicity testing including their potential uses in biocompatibility assessment.

Keywords: Genotoxicity, medical devices, risk assessment, chemical characterization, biocompatibility

1. Introduction

The EN ISO 10993-1:2020 defines medical device as "any instrument, apparatus, implement, machine, appliance, implant, reagent for *in vitro* use, software, material or other similar or related article, intended by the manufacturer to be used, alone or in combination, for human beings, for one or more of the specific medical purposes of diagnosis, prevention, monitoring, treatment, or alleviation of the disease; diagnosis, prevention, monitoring, treatment, or alleviation of or compensation of an injury; investigation, replacement, modification, or support of the anatomy or physiological process; supporting or sustaining life; control of conception; disinfection of medical devices; providing information by means of an *in vitro* examination of specimens derived from the human body do not achieve its primary intended action by pharmacological, immunological or metabolic means, in or on the body, but which may be assisted in its intended function by such means. Medical devices include dental devices" [1]. Medical devices are categorized according to nature of body contact (non-contacting, surface contacting, external contacting, and implant medical devices) and duration of body contact [limited exposure (<24 hours), prolonged exposure (>24 hours and <30 days) and permanent exposure (> 30 days)]. Risk assessment of medical devices is an important step before marketing in many regulated countries. Risk assessment and risk management is performed in line with the ISO 14971 [2]. Some of the major components of risk assessment are related to biological and chemical risks, which are addressed in ISO-10993, Parts 1 through 20 [1]. Genotoxicity testing of medical devices is covered in ISO 10993, Parts 3 [3] and 33 [4]. Assessment of genotoxicity associated with leachable and extractables from medical devices is covered in ISO 10993, Parts 17 [5] and 18 [6].

ISO-10993, Part 1 [1], classifies medical devices for evaluating biological and chemical risks based on the site and duration of contact in patients. Devices with a higher duration of patient contact and/or with a more invasive site of contact are classified under a higher risk category, which involves elaborate risk evaluation. As per ISO-10993, Part 1 [1], evaluation of genotoxicity endpoints is not required for all classes of medical devices. For instance, the following devices do not generally require genotoxicity evaluation:

1. Surface contact devices in contact with intact skin for any contact duration (e.g., gloves, bandages etc.).
2. Surface contact devices in contact with mucous membrane, with a contact duration of less than 30 days (e.g., oral care devices, urinary catheters, devices used in dental surgery etc.)
3. Surface contact devices in contact with breached or compromised skin, with a contact duration of less than 30 days (e.g., wound dressings, occlusive patches etc.).

4. External device having indirect contact with the blood path, with a contact duration of less than 30 days (e.g., IV sets etc.).
5. External device in contact with tissue, bone, or dentin, with a contact duration of less than 24 hours (e.g., endoscopes etc).
6. Implant devices in contact with bone or dentin, with a contact duration of less than 24 hours (e.g., intra cranial electrodes etc).

Any device that does not fall into the above-mentioned categories require a genotoxicity risk assessment. ISO-10993, Part 1 [1] also states that if devices contain carcinogens, mutagens, or reproductive toxins (CMR), they are subjected to risk assessment. To the best of our knowledge, this is the first review to comprehensively analyse the current regulatory aspects, understanding of genotoxicity testing, risk assessment for medical devices. It also addresses differences in the genotoxicity testing of pharmaceuticals and agrochemicals compared with that of medical devices. Further, the review also identifies and highlights the gaps for genotoxicity risk assessment of medical devices, which are not adequately addressed in the ISO standards and suggests possible ways to address them.

Genotoxicity risk assessment is performed along with risk assessment of other toxicity endpoints. This review will exclusively focus on the genotoxicity testing of medical devices and the subsequent risk assessment. The complete process of risk assessment of medical devices is examined in a systematic stepwise manner as follows:

1. Evaluation of the materials of construction
2. Extractable and leachable studies with risk assessment
3. Biocompatibility studies

2. Evaluation of Materials of Construction (Hazard-based risk assessment)

The first step in risk assessment of medical devices is to gather all the chemical information related to their configuration and composition and the materials used for their construction. This includes the main components of construction as well as other additives, processing aids, colorants, residual chemicals such as sterilizing agents, residual monomers, and other potential materials involved [6]. Once the medical device configuration has been established, each material of construction in direct or indirect contact with the patient needs to be qualitatively and quantitatively described; and its

intended interaction with body tissues and fluids must be established [1,6]. The hypothetical worst-case chemical release is established by assuming that all these materials are released into the patient's body. Thus, all material composition is screened for genotoxic potential. In terms of genotoxicity risk assessment, material composition is unlikely to provide much information, as no genotoxic materials are intentionally added to the device. Generally, some of the impurities in the components, residual monomers [7-11], residual catalysts [12,13], residual sterilizing agents [14,15] or some colorants [16-20] are likely to show some genotoxicity effects. Further, genotoxicity data may not be available for novel materials used in devices and these must be specifically investigated.

Material composition analysis sets the scene for the subsequent steps of genotoxicity risk assessment. For example, polymeric devices may contain residual monomers, many of which are genotoxic. Similarly, metallic implants containing chromium may release certain chromium ions, which may cause genotoxicity [21-25]. The information obtained by evaluating the materials of constructions, can thus be sufficient to identify the genotoxic hazards of using the device (see ISO 10993-1[1]).

Medical device manufacturers must obtain qualitative and quantitative compositional information on the materials of construction, from the suppliers of all starting materials and additives [6]. Qualitative information about any additional processing additives such as mould release agents, can also be obtained from the appropriate members of the manufacturing chain, including convertors and component suppliers [6]. All chemical constituents are then screened for genotoxic potential using reputable databases like the European Chemical Agency (ECHA) [26], LeadScope [27], Hazardous Substance Database (HSDB) [28]. If all the materials are non-genotoxic, genotoxicity risk assessment is completed and documented. If any chemical constituent has genotoxic potential or does not have genotoxicity data, Extractable and/or Leachable studies are recommended.

3. Extractable and leachable studies (Exposure based risk assessment)

Extractable and/or leachable studies with risk assessment are carried out if genotoxic chemicals are used in the manufacture of devices, or if complete qualitative and quantitative information about the materials of construction is not available. Extractable and/or leachable studies provide qualitative and quantitative estimation of the chemicals extracting (worst case) or leaching (clinical use) out from the device during its use. These extracting/leaching chemicals are then risk assessed for all toxicology endpoints including the genotoxicity endpoints.

According to the ISO 10993-18 [6], the extent of chemical characterization required depends on (a) what is known about the material formulation, (b) what nonclinical and clinical safety and toxicological data exist and (c) on the nature and duration of body contact with the medical device. At the minimum,

the characterization should address the constituent chemicals of the medical device and the possible residual process aids or additives used in its manufacture. Implant devices require thorough chemical characterization, compared with short term surface contact devices such as ECG electrodes because, exposure with invasive devices is greater compared to that with non-invasive devices.

3.1 Analysis for material composition

Chemical analysis of medical devices to understand their composition is generally not required for risk assessment purposes. This is mostly for regulatory purposes, such as EU MDR Annex 1 (Section 10.4) which mandates the disclosure of carcinogen, mutagen, or reproductive toxins (CMR) or endocrine disruptor (ED) materials in excess of 0.10% w/w [29]. The EU MDR further states that if these levels are greater than 0.10%, the risk assessment needs to be documented [29]. It is highly unlikely that mutagenic compounds will be present at levels greater than 0.10% w/w. Fourier transform infrared spectroscopy (FTIR) or X-ray diffraction (XRD) is routinely used to identify the materials in medical devices. However, these methods cannot be used for the quantitative estimation of chemicals. For quantifying inorganic materials, the device can be digested in nitric acid or other similar acids and the quantities of elements can then be measured using inductively coupled plasma mass spectroscopy (ICP-MS). Notably, this method will identify only the elements and cannot identify compounds. Organic materials in medical devices are more difficult to quantify. We have used thermo-desorption spectroscopy (TDS) coupled with gas chromatography mass spectroscopy (GC-MS) to qualify and semi-quantify organic materials in medical devices., in line with ISO 10993-18:2020 [6].

3.2 Extractable studies for worst-case extractables

This is a crucial step for genotoxicity risk assessment. Depending on the nature and duration of body contact, different extraction strategies are considered. For permanent and prolonged contact devices, exhaustive extraction is recommended. For limited contact devices, exaggerated extraction is recommended. Generally, exaggerated extraction can be performed for all devices except permanent implants, for which exhaustive extraction is the most preferred. All extracts are analysed for volatile organic compounds (VOC), semi-volatile organic compounds (SVOC), non-volatile organic compounds (NVOC) and elements using head space gas chromatography mass spectroscopy (HS/GC-MS), GC-MS, liquid chromatography mass spectroscopy (LC-MS) and ICP-MS. Care is taken to not degrade the device while extraction [6].

Exaggerated extraction is intended to result in the release of a greater number or quantity of chemical constituents compared to that generated under the clinical conditions of use. This is achieved using harsher vehicles and elevated temperatures. The commonly used exaggerated extraction conditions

include a combination of polar, semi-polar, and nonpolar vehicles with extraction temperatures up to 70 °C, generally with regular shaking or recirculation [30]. Notably, the solvents used must be compatible with the device material, without degrading the device.

Exhaustive extraction is a multi-step extraction process conducted until the amount of material extracted in a subsequent extraction step is less than 10 % by gravimetric analysis (or by any other means) of that determined in the initial extraction step. Extracts from all extraction cycles are pooled and tested. Exhaustive extraction involves the combination of polar, semi-polar, and nonpolar vehicles with extraction temperatures of up to 70 °C, with regular shaking or recirculation [30].

After extraction studies, all extracted compounds are subjected to risk assessment for all toxicity endpoints, including genotoxicity. All extracted compounds are risk assessed for genotoxicity potential based on literature from reputable sources and by demonstrating that these do not pose genotoxicity hazards. Where data is not available for identifiable chemical structures, attempts are made to obtain genotoxicity alerts using the qualitative structural activity relationship (QSAR). If still unable to demonstrate lack of genotoxicity, the concept of threshold of toxicological concern (TTC) is used (please see the section 3.4). Similarly, TTC can be used to support unidentified compounds.

A device is considered not to have genotoxic potential, if:

1. No genotoxic chemicals are extracted out.
2. Levels of 'unknown chemicals' extracted out are below the TTC for genotoxic compounds.
3. 'Extractables with QSAR alerts for genotoxicity,' are below the TTC for genotoxic compounds.

If the device does not show genotoxicity potential based on extraction studies, genotoxic risk assessment is considered complete and no further work is necessary. If extraction studies do not show a clear non-genotoxic outcome, further 'simulated use' or 'accelerated' extraction studies are performed to refine the exposure assessment.

3.3 Simulated or accelerated extraction (leachability studies) to establish clinical use extractable profiles

Simulated or accelerated extraction studies are conducted to establish clinically relevant extractables that would be expected to be released during the clinical use of the device. Depending on the nature and duration of body contact, different extraction strategies are considered. Generally, an ethanol/water mixture or saline adjusted to appropriate pH are considered excellent vehicles for

simulated extractions. Accelerated extraction is achieved by increasing the temperature without degrading the device and by including recirculation, agitation, or sonication. All extracts are analysed for Volatile Organic Compounds (VOCs), Semi Volatile Organic Compounds (SVOCs), Non-Volatile Organic Compounds (NVOCs), and elements using, Head Space -Gas Chromatography/Mass Spectrometry (HS-GC/MS), Gas Chromatography -Mass Spectrometry (GC/MS), Liquid Chromatography- Mass Spectrometry (LC/MS), and ICP -MS, respectively.

All extracted compounds are risk assessed as described in Section 3.2.

3.4 Application of analytical evaluation threshold (AET) and threshold of toxicological concern (TTC) in genotoxicity risk assessment

Usually, several compounds are identified in extractable and leachable (E&L) studies, and it is difficult to identify and quantify all of them. To minimize the work of analytical chemists and toxicologists without compromising scientific integrity, compounds present at extremely low levels (below a defined threshold, such as TTC) that have no appreciable risks to human health, are not reported. An essential component of an E&L testing study includes setting an analytical evaluation threshold (AET), which was introduced in the recent update of ISO 10993-18 [6]. AET is defined as the threshold at or above which a chemist shall begin to identify a particular leachable and/or extractable and report it for potential toxicological assessment. The AET is a relative value based on the (i) Safety concern threshold (SCT), (ii) duration and frequency of patient contact, and (iii) analytical techniques/methods used. The AET is determined during extractable studies and is applied to both extractables and leachables. The AET depends on the quantitative approach used - formal and relative quantitation. In formal quantitation, which is the preferred approach, compounds are quantified using high purity analytical standards (reference compounds) at a series of concentrations. However, when standards are not available, relative quantitation is employed. In relative quantitation, the compounds in a sample are compared against surrogate standards; the accuracy of this method depends on the surrogate standards used. The type of quantification used reflects the uncertainty factors used in AET calculations and is beyond the scope of this manuscript.

$$AET = DBT\ (\mu g/day) \times (A / (B \times C \times D)) \div UF$$

Dose based threshold (DBT) = TTC or SCT
A = number of medical devices extracted
B = extract volume
C = number of medical devices that contact the body
D = dilution factor
UF = uncertainty factor of analytical methods

The TTC approach was initially developed for the toxicological risk assessment of impurities present at low levels, whose toxicity data are not available [31]. This concept is applied to impurities in food contact materials, drugs, and currently, medical devices. Notably, TTC cannot be used for cohorts of concern, such as very potent carcinogens and metals, and for compounds for which toxicology data are available. TTC is based on the lifetime exposure of chemicals that would cause cancer in no more than 1 of 10^5 patients over their lifetime. Therefore, the levels of chemicals supportable using TTC will depend on patient exposure. ISO 21726 (ISO/TS 21726, 2019) indicates the TTC levels for various classes of devices – device contact duration of less than 30 days, 120 µg/day; 30 days to 1 year, 20 µg/day; and > 1 year, 1.5 µg/day [32].

3.5 Qualitative Structure activity relationship (QSAR)

QSAR is routinely used to determine toxicological alerts of known chemicals without sufficient toxicological data, found in extractable and leachable studies. QSAR is an *in-silico* method used for predicting the toxicity of chemical substances based on their chemical structures. In the past few years, QSAR has been efficiently utilised for predicting genotoxicity and other toxicity endpoints. Some commonly used QSAR tools for genotoxicity alerts are the Derek Nexus (Lhasa limited, UK; rule-based QSAR), Sarah Nexus (Lhasa limited, UK; statistics-based QSAR), CASE ultra (MultiCASE Inc., USA; statistics-based QSAR), Leadscope model applier, LSMA (Leadscope Inc., USA; statistics-based QSAR), and others. There is no specific use of QSAR in E&L analysis of medical devices. It is being mentioned here for the sake of completion as it may be useful in identifying some degradation products especially from drug device combinations. If unknown compounds have genotoxic alerts, then supportable levels in extracts are limited by the corresponding TTC, depending on the duration of contact.

4. Current biocompatibility tests for evaluating genetic toxicology endpoints

Biocompatibility tests for genotoxicity endpoints are required only when the devices cannot be adequately evaluated by chemical characterization, i.e., by evaluation of the construction materials and by extractable or leachable studies.

Genotoxicity tests are designed to evaluate two major endpoints - gene mutations and chromosomal damage. No single assay can detect these genotoxicity endpoints because chemicals act via diverse genotoxic mechanisms. Therefore, genotoxicity evaluations are performed using a battery of tests which employ both bacterial and mammalian test systems.

4.1 Genetic toxicology testing strategy

The strategy for genotoxicity testing is described in ISO 10993, Part 3. In the first instance, two *in vitro* tests are conducted – test for gene mutations (Ames test) and test for chromosomal aberrations (the chromosomal aberration, *in vitro* micronucleus, or mouse lymphoma assay). These *in vitro* genetic toxicology tests are conducted as per the respective OECD guidelines with appropriate modifications to accommodate medical devices.

The Ames test must be conducted on two extracts (polar and non-polar) [30], using all five *Salmonella typhimurium* (i.e. TA98, TA100, TA1535, TA1537, and TA102) or *E. coli* strains (e.g. WP2 uvrA or WP2 uvrA (pKM101)), both in the presence and absence of metabolic activation [33]. Chromosomal aberration (OECD 474), *in vitro* micronucleus (OECD 487), and mouse lymphoma (OECD 490) assays are also conducted using two extracts (polar and non-polar) [30] and three treatment regimens (short treatment +/- metabolic activation and long treatment without metabolic activation). The highest dose tested in the *in vitro* assays is the neat extract; if the device is a soluble chemical, the top dose specified in OECD guidelines is followed.

If both *in vitro* tests are clearly negative, the device is considered non-genotoxic, and no further evaluations are necessary. If one or two of the tests are positive, further stepwise investigations are carried out.

A. Investigating whether the results obtained are relevant to the clinical use of the medical device:

    a. Identifying whether any confounding factors resulted in a positive response (e.g., non-physiological conditions, interaction of the test article with culture medium, cytotoxicity, or others).

    b. Identifying whether any metabolic effects contributed to a positive response (e.g., the nature of S9, unique metabolites formed with S9 only).

    c. Identifying whether any impurities in the devices caused the positive response (e.g., some DNA binding colorants or residual monomers can cause genotoxicity).

B. Investigating the weight of evidence (WoE) along with the mechanism of action (MoA) leading to *in vitro* positive results:

a. Direct DNA reactive and indirect DNA reactive modes of action (e.g., materials releasing reactive oxygen species can cause indirect DNA damage)

b. Aneuploidy and polyploidy issues

C. Decision point and risk assessment: Decision point and risk assessment: At this point, it is determined if the positive results of *in vitro* assays are relevant to clinical use. If the positive response is irrelevant to the clinical use or due to other confounding factors, the device is considered not to present a genotoxic risk during the intended clinical use and the appropriate risk assessment is documented. If the positive genotoxic response is due to impurities, the risk may be managed by removing these impurities. Genotoxic responses due to indirectly acting DNA damaging agents can be risk managed. Similarly, NOEL can be used to risk manage aneuploidy agents. If the positive genotoxicity response cannot be attributed to the above, further investigations are necessary.

D. Selecting and running additional *in vitro* and/or *in vivo* tests: *In vivo* bone marrow micronucleus or cytogenetics and transgenics can be used for further investigating the mechanism. Single cell gel electrophoresis or comet assay can also be used to detect DNA damage [34].

4.2 Genetic toxicology tests

The general principles and requirements of commonly used *in vitro* genetic toxicology tests are described below.

1. Extract preparation: Polar and non-polar extracts, either 3 cm2/mL (> 1 mm thick) or 6 cm2/mL (< 1 mm thick), are prepared at 50 °C for 72 hours, 70 °C for 24 hours, 37 °C for 72 hours, or 121 °C for 1 hour as per ISO 10993-12.
2. Top dose: The top dose used for genetic toxicology testing is the neat extract, unless limited by cytotoxicity or precipitation. A top dose is usually sufficient, but in our laboratory, we use two lower doses in addition to the top dose, in order to identify a non-cytotoxic dose. While medical device extracts may be largely non-genotoxic, all neat extracts need not be non-cytotoxic. In such cases, the inclusion of additional doses helps in identifying a non-cytotoxic concentration which can be evaluated for genotoxicity.
3. Bacterial gene mutation assay. The Ames *Salmonella*/microsome mutagenicity assay (*Salmonella* test; Ames test) is a short-term bacterial reverse mutation assay specifically

designed to detect a wide range of chemical substances that can cause genetic damage leading to gene mutations.

4. Mouse lymphoma gene mutation assay: This test has the potential to detect mutagenic and clastogenic events at the thymidine kinase (tk) locus in L5178Y mouse lymphoma tk (+/-) cells by measuring resistance to the lethal nucleoside analogue, triflurothymidine (TFT).
5. *In vitro* chromosome aberration test: This test has the potential to detect clastogenic events in cultured mammalian cells.
6. *In vitro* micronucleus test: This test has the potential to detect clastogenic and aneugenic events in cultured mammalian cells.

5. Recent advances in genetic toxicology testing with potential uses in biocompatibility assessment

Recently, new assays are being explored for inclusion in the regulatory testing of chemicals and medical devices for genotoxicity. These recent advances are generally focussed on developing unique endpoints that are specific for medical device genotoxicity (such as site of contact genotoxicity) and assays that are quicker, clinically more relevant for devices and reduce the use of animals in biocompatibility testing. Some of the following assays have been used for the testing of chemicals and their application may be extended to medical devices. It should be noted that animal studies for demonstrating genotoxicity are currently not required except in extremely rare circumstances (Section 5.2.1 of ISO10993-3, 2014). The following subsections are mostly of academic interest in terms of method development and application of the 3Rs principle in biocompatibility testing.

5.1 Combined genetic toxicology and systemic toxicity tests

In order to prevent any additional test for genotoxicity, genetic toxicology endpoints can be integrated with acute or repeated dose systemic toxicity studies. Generally, bone marrow micronucleus and/or comet assays for various target organs can be integrated with systemic toxicity studies [35,36]. For medical devices that require both systemic toxicity and genetic toxicity endpoint assessments, data on *in vivo* genotoxic potential of the extract can be additionally generated using the same set of animals, by collecting major target organs post treatment. For systemic toxicity tests conducted via implantation [37], in addition to bone marrow micronucleus and comet assays on various target organs, the 'site of contact genotoxicity' can also be investigated [38]. Genetic toxicology endpoints can also be combined with implantation studies [39].

5.2 Comet assay to evaluate site of contact genotoxicity in implants

We have recently shown that comet assay can be used to detect the site of contact genotoxicity associated with implants that do not show genotoxicity using the currently recommended biocompatibility tests. Site of contact genotoxicity is currently not included in the battery of tests for medical implants but may be a potentially useful endpoint to assess for implant devices [38,39]. This can be a useful option for studying genotoxic potential of implantable medical devices during their intended clinical use, without using additional animals.

5.3 Other genetic toxicology assays that has been applied for medical devices

Other assays that have been applied to study genotoxicity of medical devices or novel materials are comet assay [40, 41], and 3D tissues [42-44]. Developing adverse outcome pathways (AOPs|) for DNA damage is gaining impetus and is a subject of growing research interest. An AOP "describes a sequence of events commencing with the initial interaction(s) of a stressor with a biomolecule within an organism that causes a perturbation in its biology (i.e., molecular initiating event, MIE), which can progress through a dependent series of intermediate key events (KEs) and culminate in an adverse outcome (AO) considered relevant to risk assessment or regulatory decision making" [45-53]. Gamma H2AX staining has also been used to investigate genotoxicity of biomaterials [54-56].

6. Discussion

Unlike pharmaceuticals and agrochemicals, where genotoxic responses are frequently observed, it is exceedingly rare with medical devices. In our own experience of having tested over five hundred different medical devices over the past ten years, we have observed very few positive genotoxic responses. This is because medical devices are manufactured using carefully selected raw materials that have excellent safety profiles. No genotoxic materials are intentionally added to medical devices. Nevertheless, genotoxicity may arise from various sources in device manufacture, but this is generally not due to the raw materials used. The major sources of genotoxins are the residual monomers [57-65], degradation products [66,67], metallic catalysts and metallic nanoparticles [68], colourants, and additives [69,70] that are used at very low levels during the manufacturing process.

As many medical devices are made of polymers, the genotoxicity caused by residual monomers is of primary concern. Polymers are predominantly made from monomers derived from the petrochemical industry including ethylene, propylene, styrene, terephthalic acid, ethylene oxide, caprolactam, adipic acid, and hexamethylene diamine as well as others such as acrylates. Some of these monomers, especially, the acrylates are known to be genotoxic [57,58,71]. Hence, it is important to estimate the risk caused by residual monomers in polymers.

Some medical devices can undergo degradation within the body (e.g., sutures). Although some medical devices are intended to degrade over a period (sutures), others (Implants) undergo degradation due to wear and tear. Degradation products from polymers can release monomers that may be genotoxic. Wear and tear degradation particles from devices such as artificial joints can also release metallic particles or even nanoparticles, which can be genotoxic [66,68]. For devices undergoing degradation, genotoxicity must be assessed before and after degradation.

Metals are another subset of materials that can cause genotoxicity. Metallic ions can leach from medical implants made of titanium, cobalt chromium, or stainless steel. Generally, the levels of these ions are too low to cause any toxicity. Highly reactive metals are used as catalysts in the manufacture of polymers. These residual catalysts in polymer medical devices can cause genotoxicity. Oxidative DNA damage, interference with DNA repair, and deregulation of cell proliferation are the primary mechanisms associated with the genotoxic and carcinogenic effects of metals [72,73].

Nanoparticles are another important contributor to the genotoxicity of medical devices. With increasing use of nanomaterials in medical devices, genotoxicity risk assessment of such devices is of special interest. Nanomaterials are known to cause genotoxicity via different mechanisms. Certain nanomaterials can cross the cell membrane, enter the nucleus, and interact with nuclear DNA and proteins. Direct contact with DNA can also occur during cell division when the nuclear envelope disappears. Nanoparticles can generate free radicals following interaction with cell constituents of the same scale and can induce DNA lesions or affect chromosome segregation during mitosis, resulting in perturbation of cell division and disorganization of cell-trafficking. DNA damage can also result from indirect mechanisms involving prooxidative effects or DNA repair inhibition. Not all DNA damage caused by nanoparticles results in carcinogenesis.

Genotoxicity testing of nanoparticles presents several challenges. It is generally accepted that nanoparticles do not enter bacterial cells. Therefore, *in vitro* mammalian gene mutation assays are preferred over the Ames test. The commonly used *in vitro* tests for detecting genotoxicity from nanomaterial-containing medical devices include the mouse lymphoma assay, *in vitro* micronucleus assay, and the *in vitro* comet assay. *In vivo* genotoxicity tests must be designed based on toxicokinetics data to identify target organs [74,75]. It is very difficult to analyse the levels and size of nanoparticles in tissues using standard analytical techniques. Single particle ICP-MS (spICP-MS) [76,77] and asymmetrical field-flow fractionation (AF4) coupled with ICP-MS (AF4-ICP-MS) are generally used to measure the size and content of various nanoparticles in tissues [78]. The comet assay is especially useful to study DNA damage in target tissues [74]. Metallic nanomaterials used in medical devices such as nano-silver [79], gold [80], and nickel [81,82], have been shown to be genotoxic. Currently,

there are no clearly defined strategies for assessing the genotoxicity of nanomaterial containing devices. These devices must be assessed on a case-by-case basis by experts in the field.

In terms of testing strategy, there is a definite disconnect between the genotoxicity testing of medical devices and that of chemicals/pharmaceuticals. While all pharmaceuticals and agrochemicals require testing for genetic toxicity, only certain classes of medical devices require genetic toxicity testing. Interestingly, that even permanent skin contacting devices are exempted from genotoxicity testing. In terms of test selection, genotoxicity test requirements for pharmaceuticals as per ICH S2(R1) [40] comes with two options: the first being a combination of the bacterial reverse mutation test, an *in vitro* cytogenetic test and *in vivo* micronucleus test and the second includes a combination of the bacterial reverse test and an *in vivo* test which combines the micronucleus assay and comet assay. Even with pesticides, requirement of a combination of *in vitro* and *in vivo* tests is recommended. However, where possible, integration of *in vivo* genotoxicity endpoints into repeated dose toxicity studies is also accepted. In case of medical devices, a combination of the bacterial reverse mutation test and an *in vitro* cytogenetic assay or the *in vitro* gene mutation assay is recommended. *In vivo* studies are generally not recommended as a routine. *In vivo* studies are considered when the studies on the genotoxic mechanism and pharmacokinetics are needed.

Genetic toxicology tests for pharmaceuticals and agrochemicals are designed to evaluate genotoxicity hazards. This is achieved by testing chemicals at the recommended top dose, considering the appropriate cytotoxicity, or presence of precipitates. For an acceptable negative result, it is important to demonstrate that the chemical was tested by fulfilling the above conditions. However, the definition of an acceptable top dose is not well defined in the case of medical devices. The top dose is the neat extract prepared as per ISO 10993-12 [30], which is a mixture of chemicals. Additionally, extract preparation differs for different classes of devices and is dependent on the thickness of the smallest part of the device and its materials (e.g., membranes are extracted differently from solid devices). Therefore, different top doses may be required for different devices. The OECD test guidelines recommend testing of complex mixtures at higher concentrations to maximise the exposure of substances present at low concentrations. All testing of medical devices is carried out with extracts made as per the ISO 10993-12 guidelines [30], which are mixtures of various chemicals. Currently, there are no provisions to increase the top dose for genetic toxicology assays.

The use of higher surface area for extraction can be a useful option to increase the concentration of genotoxins in the extract. ISO 10993-12 suggests the use of 3 or 6 cm^2/mL or 0.1 g/mL as the extraction ratio for biocompatibility studies [ISO 10993-12:2012]. The extracts thus obtained may contain genotoxins (if any) that are way too less to be detected by the currently used assays. Use of higher

surface area to volume ratio may be used, however, more research work is necessary. In addition, concentrating the extracts, as in the case of the Japanese standards (MHLW,2020) would increase the non-volatile residues but some potentially genotoxic, volatile and semi-volatile chemicals, may be missed out [70].

In this manuscript, we have reviewed the current regulatory understanding regarding genotoxicity risk assessment of medical devices. From this review we conclude that more rigorous extract preparation for genotoxicity testing (as described above) and evaluation of genotoxicity endpoints for all medical devices may be required. A well-designed, robust chemical characterization can replace the entire battery genotoxicity testing, if they demonstrate absence of any genotoxins in the medical device extract. The strategies mentioned above can provide a more meaningful approach to the genotoxicity evaluation of medical devices, prospectively.

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