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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

99   intended interaction with body tissues and fluids must be established [1,6]. The hypothetical worst-
100   case chemical release is established by assuming that all these materials are released into the patient's
101   body. Thus, all material composition is screened for genotoxic potential. In terms of genotoxicity risk
102   assessment, material composition is unlikely to provide much information, as no genotoxic materials
103   are intentionally added to the device. Generally, some of the impurities in the components, residual
104   monomers [7-11], residual catalysts [12,13], residual sterilizing agents [14,15] or some colorants [16-
105   20] are likely to show some genotoxicity effects. Further, genotoxicity data may not be available for
106   novel materials used in devices and these must be specifically investigated.

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

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

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

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

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

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

135 **3.1 Analysis for material composition**

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

150 **3.2 Extractable studies for worst-case extractables**

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

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

163 include a combination of polar, semi-polar, and nonpolar vehicles with extraction temperatures up to
164 70 °C, generally with regular shaking or recirculation [30]. Notably, the solvents used must be
165 compatible with the device material, without degrading the device.

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

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

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

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

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

186

187 **3.3 Simulated or accelerated extraction (leachability studies) to establish clinical use extractable**
188 **profiles**

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

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

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

200 **3.4 Application of analytical evaluation threshold (AET) and threshold of toxicological concern (TTC)**
201 **in genotoxicity risk assessment**

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

219 AET = DBT (μg/day) x (A/ (B x C x D)) ÷ UF

220 Dose based threshold (DBT) = TTC or SCT
221 A = number of medical devices extracted
222 B = extract volume
223 C = number of medical devices that contact the body
224 D = dilution factor
225 UF = uncertainty factor of analytical methods
226

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:

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

288   b. Aneuploidy and polyploidy issues

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

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

302   **4.2 Genetic toxicology tests**

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

305   1. Extract preparation: Polar and non-polar extracts, either 3 cm2/mL (> 1 mm thick) or 6
306   cm2/mL (< 1 mm thick), are prepared at 50 °C for 72 hours, 70 °C for 24 hours, 37 °C for 72
307   hours, or 121 °C for 1 hour as per ISO 10993-12.
308   2. Top dose: The top dose used for genetic toxicology testing is the neat extract, unless limited
309   by cytotoxicity or precipitation. A top dose is usually sufficient, but in our laboratory, we use
310   two lower doses in addition to the top dose, in order to identify a non-cytotoxic dose. While
311   medical device extracts may be largely non-genotoxic, all neat extracts need not be
312   non-cytotoxic. In such cases, the inclusion of additional doses helps in identifying a non-
313   cytotoxic concentration which can be evaluated for genotoxicity.
314   3. Bacterial gene mutation assay. The Ames *Salmonella*/microsome mutagenicity assay
315   (*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

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

353 **5.3 <u>Other genetic toxicology assays that has been applied for medical devices</u>**

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

362 # 6. Discussion

363 Unlike pharmaceuticals and agrochemicals, where genotoxic responses are frequently observed, it is
364 exceedingly rare with medical devices. In our own experience of having tested over five hundred
365 different medical devices over the past ten years, we have observed very few positive genotoxic
366 responses. This is because medical devices are manufactured using carefully selected raw materials
367 that have excellent safety profiles. No genotoxic materials are intentionally added to medical devices.
368 Nevertheless, genotoxicity may arise from various sources in device manufacture, but this is generally
369 not due to the raw materials used. The major sources of genotoxins are the residual monomers [57-
370 65], degradation products [66,67], metallic catalysts and metallic nanoparticles [68], colourants, and
371 additives [69,70] that are used at very low levels during the manufacturing process.

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

378   Some medical devices can undergo degradation within the body (e.g., sutures). Although some

379   medical devices are intended to degrade over a period (sutures), others (Implants) undergo

380   degradation due to wear and tear. Degradation products from polymers can release monomers that

381   may be genotoxic. Wear and tear degradation particles from devices such as artificial joints can also

382   release metallic particles or even nanoparticles, which can be genotoxic [66,68]. For devices

383   undergoing degradation, genotoxicity must be assessed before and after degradation.

384   Metals are another subset of materials that can cause genotoxicity. Metallic ions can leach from

385   medical implants made of titanium, cobalt chromium, or stainless steel. Generally, the levels of these

386   ions are too low to cause any toxicity. Highly reactive metals are used as catalysts in the manufacture

387   of polymers. These residual catalysts in polymer medical devices can cause genotoxicity. Oxidative

388   DNA damage, interference with DNA repair, and deregulation of cell proliferation are the primary

389   mechanisms associated with the genotoxic and carcinogenic effects of metals [72,73].

390   Nanoparticles are another important contributor to the genotoxicity of medical devices. With

391   increasing use of nanomaterials in medical devices, genotoxicity risk assessment of such devices is of

392   special interest. Nanomaterials are known to cause genotoxicity via different mechanisms. Certain

393   nanomaterials can cross the cell membrane, enter the nucleus, and interact with nuclear DNA and

394   proteins. Direct contact with DNA can also occur during cell division when the nuclear envelope

395   disappears. Nanoparticles can generate free radicals following interaction with cell constituents of the

396   same scale and can induce DNA lesions or affect chromosome segregation during mitosis, resulting in

397   perturbation of cell division and disorganization of cell-trafficking. DNA damage can also result from

398   indirect mechanisms involving prooxidative effects or DNA repair inhibition. Not all DNA damage

399   caused by nanoparticles results in carcinogenesis.

400   Genotoxicity testing of nanoparticles presents several challenges. It is generally accepted that

401   nanoparticles do not enter bacterial cells. Therefore, *in vitro* mammalian gene mutation assays are

402   preferred over the Ames test. The commonly used *in vitro* tests for detecting genotoxicity from

403   nanomaterial-containing medical devices include the mouse lymphoma assay, *in vitro* micronucleus

404   assay, and the *in vitro* comet assay. *In vivo* genotoxicity tests must be designed based on toxicokinetics

405   data to identify target organs [74,75]. It is very difficult to analyse the levels and size of nanoparticles

406   in tissues using standard analytical techniques. Single particle ICP-MS (spICP-MS) [76,77] and

407   asymmetrical field-flow fractionation (AF4) coupled with ICP-MS (AF4-ICP-MS) are generally used to

408   measure the size and content of various nanoparticles in tissues [78]. The comet assay is especially

409   useful to study DNA damage in target tissues [74]. Metallic nanomaterials used in medical devices

410   such as nano-silver [79], gold [80], and nickel [81,82], have been shown to be genotoxic. Currently,

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

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

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

440   The use of higher surface area for extraction can be a useful option to increase the concentration of
441   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
442   ratio for biocompatibility studies [ISO 10993-12:2012]. The extracts thus obtained may contain
443   genotoxins (if any) that are way too less to be detected by the currently used assays. Use of higher

444 surface area to volume ratio may be used, however, more research work is necessary. In addition,

445 concentrating the extracts, as in the case of the Japanese standards (MHLW,2020) would increase the

446 non-volatile residues but some potentially genotoxic, volatile and semi-volatile chemicals, may be

447 missed out [70].

448 In this manuscript, we have reviewed the current regulatory understanding regarding genotoxicity risk

449 assessment of medical devices. From this review we conclude that more rigorous extract preparation

450 for genotoxicity testing (as described above) and evaluation of genotoxicity endpoints for all medical

451 devices may be required. A well-designed, robust chemical characterization can replace the entire

452 battery genotoxicity testing, if they demonstrate absence of any genotoxins in the medical device

453 extract. The strategies mentioned above can provide a more meaningful approach to the genotoxicity

454 evaluation of medical devices, prospectively.

455

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