
British Standard Methods for
**Biological assessment of
dental materials**

Méthodes d'évaluation biologique des matériaux dentaires

Verfahren zur Beurteilung der biologischen Verträglichkeit von Dentalwerkstoffen

Foreword

This British Standard has been prepared under the direction of the Health Care Standards Policy Committee and is a revision of BS 5828 : 1980, which is withdrawn.

The principal changes from the previous edition are as follows.

(a) Methods of test for tissue implantation, bone implantation and cytotoxicity in vitro have been included.

(b) One of the optional methods of test for skin sensitization has been deleted.

Materials used in dentistry should not constitute a hazard to health or release, under the conditions of intended use, substances in quantities sufficient to produce such a hazard. EEC Directives and consequent national legislation concerning medicinal products require the manufacturers of dental materials to provide evidence that those of their materials that are medicinal products are biologically safe. Many dental materials are used in a manner different from that for the majority of other materials in medicine in that they are introduced into the body in an unset, or partially polymerized, state. The methods of test used to evaluate their biological safety are also different and it is largely for this reason that this standard has been prepared, detailing methods of test that are of particular relevance to dental materials.

The methods given in this standard are as far as possible in conformity with those given in BS 5736 and with other national and international standards, in particular the following.

(a) ISO/TR 7405 published by the International Organization for Standardization.

(b) DIN 13930 published by Deutsches Institut für Normung.

(c) ANSI/ADA Document no. 41a published jointly by the American National Standards Institute and the American Dental Association.

This standard gives methods to assess the biological hazard of new or modified dental materials for which adequate data are not already available. It is concerned primarily with reducing to a minimum the possibility that biologically unsatisfactory materials may be used in the treatment of patients, although tests concerned with the hazard to the

operators who may handle the materials either in the dental surgery or dental laboratory are also included. It is not intended that the test methods contained within this standard should be used routinely for batch testing in quality control.

The test methods have been chosen on the basis of their suitability as methods for evaluating the biological properties of dental materials, bearing in mind the potential risks that might arise during clinical usage. The number of tests described is limited and there may be circumstances when wider testing is indicated. It is essential to appreciate that however exhaustive the number of laboratory and animal tests performed, a material may still give occasional unwanted effects in clinical use. The tests recommended, therefore, reflect a balance between the need to minimize the risk of biological side effects against the costs involved. It is considered that a more exhaustive standard would not significantly reduce the biological risks, and may inhibit the development of dental materials that may have only a small potential market.

Guidance is given as to which tests are appropriate to the evaluation of each category of dental material. It is not intended that all designated tests are necessarily to be completed in every instance. The range of tests to be applied to each new material should be decided by a toxicologist with experience in the field of dental materials. Previous knowledge of the toxicology of chemically related materials and/or their individual constituents and the requirements of regulatory authorities should be taken into account in reaching this decision. As the interpretation of the results obtained in some of the test methods is reliant upon subjective assessment, it is essential that they are undertaken only by persons with the necessary expertise and experience in the field of biological testing relative to dentistry.

In order to minimize variation in the interpretation of results, quantitative or semi-quantitative guidelines are given where possible for the assessment of the degree of reaction.

For in vivo tests, attention is drawn to the provisions of the Animals (Scientific Procedures) Act 1986.

Compliance with a British Standard does not of itself confer immunity from legal obligations.

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Methods

1 Scope

This British Standard describes biological test methods and methods of assessment for the evaluation of the biological hazards of those dental products classified as dental materials.

Systems of classification for dental products and dental materials are given in appendices A and B respectively.

The overall assessment of the results should be carried out by a toxicologist who has appropriate chemical and biological data concerning the dental material and who is aware of the conditions of its use.

NOTE. The titles of the publications referred to in this standard are listed on the inside back cover.

2 General

2.1 Dental materials should be evaluated against the appropriate test methods as given in table 1. Where a particular box in table 1 is blank, it indicates that the test method is not considered appropriate for that particular type of material.

The dose levels used in all tests, where not indicated, are to be selected in accordance with the typical mass or volume of material that would be used in practice. Data on the typical volumes of materials are given in table 2.

2.2 Any biological hazard from dental materials depends on many factors including their use, frequency and duration of exposure, amount and identity of any substances entering the body and their biological activity.

2.3 Although this standard is concerned with the tests to be carried out on the final product, a knowledge of the chemical composition of the individual constituents and the method of manufacture of the material is important for any test selection. If commercial confidentiality precludes the release of this information to the manufacturer of the product, it is essential that the data are made available to those ultimately responsible for assessment, e.g. regulatory authorities.

2.4 The range of potential hazards to the patient and operator is wide and includes:

- (a) toxic effects upon not only the oral and dental tissues, but also other specific organs, tissues and systems, including possible mutagenic, carcinogenic and teratogenic effects;
- (b) irritation of the skin, eye and mucosal surfaces;
- (c) sensitization.

All potential biological hazards should be considered for every material. This does not imply that testing will be necessary or practicable in all cases.

2.5 The selection of appropriate biological tests should be based upon:

- (a) knowledge of the chemistry and of any biological effects of the constituents of a material, and of details of its formulation and manufacture;
- (b) reference to the categories of dental material based on the classification given in appendix B;

- (c) specific chemical analysis of the composition of the material after setting.

2.6 The material should be re-evaluated and further testing may be necessary if:

- (a) any change occurs in the source or in the specification of the constituents used in the manufacture of the material;
- (b) any change occurs in the formulation or processing of the material;
- (c) any unpredicted change occurs in the material during storage;
- (d) any change occurs in its intended use;
- (e) any suspicion arises that the material may produce adverse effects when used in man.

2.7 The following general principles should be applied to all biological safety testing of dental materials.

- (a) Testing should be performed on the material both in its freshly-mixed state and, if appropriate, in the fully-set state.
- (b) The choice of test procedures should take account of:
 - (1) the nature, degree, duration, frequency and conditions of exposure of man to the material or its constituents in normal use;
 - (2) the chemical and physical nature of the material;
 - (3) the toxicological activity of the chemicals in the formulation of the material.
- (c) Controls should be used, where appropriate.
- (d) Testing should be carried out in accordance with the principles of good laboratory practice.

NOTE. When carrying out tests involving animals, animals showing severe and enduring signs of distress and pain may need to be humanely killed.

2.8 The initial biological tests which should be considered may be broadly classified as follows:

- (a) *Cytotoxicity*. Cell culture techniques can provide a rapid and sensitive means of detecting adverse effects on cells which are produced by extractable or diffusible components of dental materials. Cytotoxic effects as demonstrated by these tests should not necessarily be taken as indicating that the product will present a hazard to health under the conditions of use. The model cavity method described in clause 11 is intended for filling materials and has been evaluated satisfactorily in inter-laboratory trials using the specified cell lines. A cytotoxicity test for general application is under consideration.
- (b) *Systemic toxicity*. Useful knowledge of the likely systemic toxicity from the accidental swallowing of dental materials can be obtained from these tests. As a guide for planning such studies, the typical volumes of materials commonly used in different procedures in dental treatment are given in table 2.
- (c) *Sensitization*. The sensitization potential of all new materials should be considered whether or not they come into direct contact with the patient's skin or

mucosa, because all materials are handled by the operators.

Limitations of existing test systems are such that tests on the set material may not be meaningful; investigations on the constituents may supply more useful information. Sensitization effects in susceptible individuals may not be detected until the material is in use.

(d) *Skin irritation and oral mucosal irritation.* The assessment of primary irritancy is relevant to most dental materials which are placed in contact with the oral mucosa and/or which are handled by the operators in normal use.

(e) *Soft tissue and bone implants.* The examination of the effects of direct contact of the material with these tissues is particularly relevant to those materials which are placed into root canals or are implanted in the jaws, and will also give information of value for the assessment of some other materials which may come into contact with tissue.

(f) *In-use test for filling materials.* This test is intended to evaluate the tissue reaction to materials when used as intra-coronal fillings. It is important to appreciate that under these conditions a tissue response may arise not only from chemical toxicity but also from

mechanical failure, thus allowing microbial microleakage, etc.

(g) *In-use test for root canal sealing materials.* This test is intended to evaluate the periapical tissue reaction to sealing materials when they are used in root fillings. It is important to appreciate that under these conditions a tissue response may arise not only from chemical toxicity but also from microbial contamination.

(h) *Mutagenicity and carcinogenicity.* The possible mutagenic and carcinogenic properties of all new materials and all new constituents of established materials have to be evaluated. The evaluation of these properties is a specialized field of study and no methods for this are included in this standard. However, there are well-established protocols for such evaluation and it is recommended that at least a reverse mutation microbial assay and an in vitro mammalian cytogenicity test are carried out, as recommended in the OECD guidelines*.

2.9 No test results can ensure freedom from biological hazard. Biological investigations should be followed by careful observations for the appearance of unexpected adverse reactions in man during early clinical use of the dental material.

* Details of these tests are given in OECD Guidelines for Testing of Chemicals, published by the Organisation for Economic Co-operation and Development, 2 Rue André-Pascal, 75775 Paris Cedex 16, France.

Table 1. Tests appropriate to types of dental material

Type of material*	Test and clause number								
	Systemic toxicity test (oral route) (3)	Skin sensitization (4)	Skin irritation (5)	Oral mucosal irritation (6)	Tissue implantation (7)	Bone implantation (8)	In-use test for		In vitro test for cytotoxicity (11)
							Dental filling materials (9)	Root canal sealing materials (10)	
Base plate resins and waxes	x	x	x	x					
Ceramics	x			x					
Dental resins and tooth materials	x	x	x	x					
Endodontic materials	x	x	x	x	x	x		x	
Filling materials	x	x	x	x	x		x		x
Impression materials	x	x	x	x					
Metals		x	x	x					
Plasters, investments and die materials		x	x						
Prophylactic/preventive materials	x	x	x	x					
Implant materials	x	x		x	x	x			
Clinical sundries	x	x		x					
Laboratory sundries		x	x						
NOTE. The tests appropriate for the various types of material are indicated by x.									
*For a more detailed classification of dental materials, see appendix B.									

Table 2. Typical volumes of materials commonly used in dental treatment

Material	Typical volume
	mL
Impression materials (average of full upper and lower impressions)	40 (range 35 to 45)
Denture base resin (full upper or full lower denture)	20
Filling materials	
Posterior, one surface	0.10
Posterior, two surfaces	0.20
Posterior, three surfaces	0.35
Anterior approximal	0.05
Lining	0.02
Base	0.10
Single root filling	0.15
Root canal sealer	0.30

3 Systemic toxicity test: oral route

3.1 Object

The object of the test is to obtain information on the systemic toxicity of a material following oral administration of a test sample for 14 days.

3.2 Animals and husbandry

3.2.1 Twenty male and 20 female albino rats aged 4 weeks to 12 weeks, at the start of the test, shall be used.

3.2.2 Ten male and 10 female rats shall be randomly assigned to each of two groups, one to be the test group and the other to be the control group. The rats shall be housed five to a cage according to sex.

3.2.3 The animals shall have free access to food and water.

3.3 Preparation of test samples

3.3.1 If the test material is a solid, grind it to a powder with a maximum particle size of 200 µm and prepare test samples for administration as specified in **3.3.2**.

NOTE. The use of diamond abrasive and/or low temperatures is recommended in order to minimize the amount of debris from the grinding medium.

If the test material is a liquid, use it directly to prepare the test samples for administration as specified in **3.3.2**.

3.3.2 Prepare a test sample for administration to each of the animals as one of the following:

- (a) a solution in distilled water;
- (b) a suspension in a non-toxic vehicle;
- (c) if both (a) and (b) are not possible, by incorporation directly into the diet.

NOTE. The physical and chemical stability and homogeneity of solutions, suspensions and diets should be established before dosing commences and then assessed at least once during the treatment period.

3.4 Test procedure

3.4.1 Preparation of animals

3.4.1.1 Acclimatize the animals in their cages for at least 5 days prior to testing.

3.4.1.2 Weigh each animal immediately before administration of the test samples and calculate the mean body mass of the animals in each group.

3.4.2 Administration of test samples

3.4.2.1 Administer a dose of the test sample to each animal of the test groups, at a level as specified in **3.4.2.2**, daily for 14 days either by oral gavage using a syringe and catheter or by dietary inclusion.

3.4.2.2 Determine the dose level from available toxicological data on the test substance or chemically related substances and from anticipated human exposure data (see table 2). For solutions and suspensions, ensure that the dose level is such that there is an acceptable safety margin over the anticipated human exposure and does not exceed 1000 mg/kg body mass per day. For dietary inclusion, ensure that the dose level does not exceed 5 % by mass of the food.

NOTE. For solutions and suspensions, the maximum dosage volume should not exceed 10 mL/kg body mass.

3.4.2.3 Administer either water or the vehicle, as appropriate, to the animals of the control groups.

3.4.3 Observation of animals

3.4.3.1 Observe the animals daily from the first day of treatment and record any signs of reaction to treatment in terms of time to onset, duration and intensity.

3.4.3.2 At 7 days and 14 days after commencing the test, weigh each animal and calculate the mean body mass of the animals in each group.

3.4.3.3 Record the food consumption for each cage at weekly intervals and calculate the group mean food consumption per day.

3.4.3.4 If any animal dies or has to be killed for humane reasons during the test, perform a detailed macroscopic examination and preserve tissue samples for post-mortem examination as described in **3.6**.

3.5 Haematological and blood biochemistry studies

3.5.1 After 14 days of exposure to the test or control materials, a sample of blood shall be taken from each animal and the haematological and blood biochemistry studies described in **3.5.2** and **3.5.3** respectively shall be carried out.

3.5.2 Haematological studies shall include at least the determination of haematocrit, haemoglobin concentration, erythrocyte count, total and differential leucocyte count, and coagulation studies.

3.5.3 Clinical biochemistry studies shall include at least one parameter of liver and kidney function: serum alanine aminotransferase and serum aspartate aminotransferase activity, and the amount of urea nitrogen, albumin, blood creatinine, total bilirubin and total serum protein present.

NOTE 1. Other evaluations which may be necessary for an adequate toxicological evaluation include the amount of calcium, phosphorus, chloride, sodium, potassium, methaemoglobin, fasting glucose, hormones, and acid/base balance, analysis of lipids and cholinesterase activity.

NOTE 2. Additional clinical biochemistry may be employed, where necessary, to extend the investigation of observed effects.

3.6 Post-mortem examinations

3.6.1 Autopsy. After carrying out the studies described in 3.5, kill the animals and conduct an autopsy. Record the macroscopic appearance of any organs, including, but not restricted to, the adrenals, heart, lungs, gastro-intestinal tract, liver, spleen, kidneys and gonads. Weigh the adrenals, gonads, kidneys and liver of each animal.

3.6.2 Histopathological examination. Remove the following tissues and place them in fixative solution for possible histological examination, if the need for this is indicated by the results of the clinical biochemistry and macroscopic studies:

- (a) adrenals;
- (b) bladder (urinary);
- (c) bone marrow (sternum);
- (d) brain;
- (e) eyes;
- (f) gonads;
- (g) accessory sex organs (epididymis, seminal vesicle, prostate, uterus);
- (h) Harderian glands;
- (i) heart;
- (j) intestines (duodenum, ileum, colon);
- (k) kidneys;
- (l) liver;
- (m) lung (whole);
- (n) lymph nodes (mesenteric);
- (o) muscle (quadriceps);
- (p) nerve (sciatic);
- (q) pancreas;
- (r) pituitary gland;
- (s) salivary glands (sub-lingual, sub-maxillary, parotid);
- (t) skin;
- (u) spleen;
- (v) stomach;
- (w) thymus;
- (x) thyroid on trachea;
- (y) any tissues of abnormal appearance.

3.7 Presentation of results

The results shall be submitted in a test report that includes a complete record of all procedures followed and any other relevant data necessary for the assessment of results as described in 3.8.

3.8 Assessment of results

3.8.1 All the information in the test report shall be taken into consideration in assessing the test results, particularly

any differences in the results between the test groups and control groups, and between the sexes.

3.8.2 The results of the assessment shall be recorded in the test report.

4 Skin sensitization tests

NOTE. Two test methods are described, although it is only necessary to evaluate materials against one of the methods. Use method 1 unless specific problems preclude its use. Both methods are known to have deficiencies in detecting reactions caused by certain skin sensitizers.

4.1 Object

The object of both tests is to assess the potential of the material under test to induce skin sensitization.

4.2 Animals and husbandry

4.2.1 A minimum of 21 (method 1) or 24 (method 2) albino guinea-pigs of either sex from a single outbred strain, weighing 350 g to 550 g at the start of the test, shall be used.

4.2.2 The animals shall have free access to food and water and shall be caged either singly or in groups.

4.3 Preparation of test material

Dissolve or suspend the test material in a non-irritant vehicle (see 4.6.1.4 and 4.7.1.3).

4.4 Preparation of animals

Clip the fur on all treatment sites and shave those for topical applications, at least 24 h before treatment.

4.5 Administration of test materials

For all intradermal injections, inject into the clipped intrascapular region of the animal, using 0.1 mL of sample per injection site.

For all topical applications, saturate a patch of filter paper or surgical gauze of the appropriate dimensions with the test material and apply the patch to the clipped and shaved skin surface under a retaining dressing, e.g. an occlusive dressing wound around the torso of the animal.

4.6 Method 1

4.6.1 Preliminary tests

4.6.1.1 The preliminary tests described in 4.6.1.2 and 4.6.1.3 are to determine the concentrations of the test material to be used in the main test described in 4.6.2.

4.6.1.2 Inject a range of concentrations of the test material intradermally into each of two animals. Record the diameter of each injection site.

After 24 h, assess the resulting dermal reactions using the gradings given in table 3.

Select for the intradermal induction phase (see 4.6.2.2) in the main test, the highest concentration that causes no more than slight erythema and does not otherwise adversely affect the animals.