5. OTHER DATA RELEVANT TO AN EVALUATION OF CARCINOGENICITY AND ITS MECHANISMS

5A. Metallic Medical and Dental Materials

5A.1 Degradation of metallic implants in biological systems

Implanted metallic materials are subject to corrosion, which can result from direct interaction with the surrounding tissue or body fluids or be the consequence of mechanical damage. The resistance of metallic biomaterials to corrosion depends on the presence of a passive, protective film of oxide covering the surface. Titanium, which appears as an active metal in the electromotive series, forms a resistant oxide, which prevents further corrosion. Stainless steel and cobalt alloys form chromium oxide films. The composition of the oxide film has an important influence on biocompatibility. Metallic biomaterials in an aqueous environment represent a system in which active and passive surfaces exist simultaneously in contact with electrolyte (Kelly, 1982). At the surface of the metal oxide film, there is a continuous process of dissolution and reprecipitation, so that the composition of the film can change even though it seems macroscopically stable. Calcium, phosphorus and sulfur have been found to be incorporated into the surface film of titanium bolts surgically implanted into human jaw bone (McQueen *et al.*, 1982). Similarly, calcium and phosphorus have been detected in the oxide film of 316L stainless steel pins and wires that had been implanted during hand surgery and maxillofacial surgery (Sundgren *et al.,* 1985).

5A.1.1 *Mechanisms of degradation*

The principal mechanisms by which surgical alloys corrode are galvanic, crevice and fretting corrosion. These types of degradation involve the release of ions. Galvanic corrosion can occur when two metallic implants of different composition or two regions of the same implant with different electrochemical properties are in contact. For example, during the development of techniques for internal fixation of fractures, plates of one material were occasionally fixed with screws of another material. In modular total hip prostheses, femoral components may have a head made of a cobalt–chromium–molybdenum (CoCrMo) alloy and a stem made of titanium–aluminium–vanadium alloy (Ti 6,4). However, as more corrosion-resistant materials have become used in such mixed metal combinations, the issue of mixed metal galvanic corrosion has become less problematic.

Crevice corrosion can occur in a confined space that is exposed to a chloride solution. Such a space can exist in a gasket-type connection between a metal and a non-metal or between two pieces of metal bolted or clamped together. Crevice corrosion involves an electrochemical reaction which may take six months to two years to develop. Crevice corrosion has been observed in implants in which metals are in contact, such as in some total hip replacement devices, screws and plates used in fracture fixation and some orthodontic appliances (Fontana & Greene, 1978).

Fretting corrosion is a phenomenon of microscopic shear motion between two surfaces. The range of motion is typically less than 100 μm. Fretting corrosion involves continuous disruption of the oxide film and the associated oxidation of exposed metal. In devices that undergo crevice corrosion, fretting corrosion may accelerate the degradation.

Wear is a form of degradation which involves the release of particulate debris. Surface damage due to wear can occur by several mechanisms. On a microscopic scale, contact between two surfaces is not across an entire area, but rather occurs at high points or local asperities. Adhesive wear occurs when asperities of two surfaces adhere to each other, as with local spot welding due to high contact stress. With sliding between the surfaces, portions of one surface are torn off. Abrasive wear involves a harder material ploughing through the surface of a softer one. With ductile materials like metals and some plastics, such ploughing results in a deep furrow with a raised ridge on either side. These ridges may subsequently cause wear of the opposite surface. Three-body wear occurs when free particles in a bearing space abrade the surfaces. They may cause abrasive wear of both, or may become embedded in the softer surface and cause abrasive wear of the counterface. Under suitable conditions, the protective oxide film can be restored following damage (repassivation).

5A.1.2 *In-vitro corrosion of dental alloys*

The expression ‘corrosion-resistant’ in connection with precious-metal or well passivated base-metal alloys would suggest that there is no corrosion at all. In fact this is not true, as is clear from the fact that patients often claim a metallic taste and mouth burning following metallic restoration in the mouth. The recommended in-vitro test for such corrosion involves immersion of specimens in a solution of 0.1 M lactic acid and 0.1 M sodium chloride (pH 2.2) at 37°C for seven days. The low pH is thought to mimic oral crevice conditions. The total amount of metal(s) released is determined by atomic absorption spectrophotometry (AAS) or mass spectrometry. The corrosion is expressed as μg metal released per cm^2 surface area per day. Typical values are given in Table 55. Because corrosion is exceptionally rapid in the first few days, these measurements are made after a four-day period of conditioning the specimens in a similar test solution. With precious-metal alloys, the ion-specific corrosion rate does not correlate with the amount of alloying elements; the base metals are released at a proportionately higher rate compared with the alloy composition. Beryllium as an alloying element considerably increases the corrosion rate of other metals such as nickel (Kappert *et al.*, 1998; see Table 55).

Table 55. Ion release from preconditioned specimens of various cast alloy types

Alloy type	Composition	Released ions	Amount of release ($\mu g/cm^2$ per day)
High gold	Au 77–87; Pt 9–19; Zn 1–2; In 0.7–1.6	In	1.97–2.88
Low gold	Au 51–57; Pd 31–38; In 8; Ga 1.5	In	0.48–3.84
		Ga	0.76–3.57
Palladium–copper	Pd 73–78; Cu 8.5–11.5; Ga 7–9; Sn 3–16	Pd	3.65–11.11
		Cu	6.03–9.98
		Ga	10.8–21.0
Palladium–silver	Pd 75–77; Ag 6–8; Ga 2–6.5; Au 5–6	Pd	0.1
		Ag	< 0.1
		Ga	1.22–2.36
Cobalt-based alloy	Co 63–65; Cr 27–29 Mo 5–7; Mn < 1	Co	0.3
Nickel-based alloy without Be	Ni 65; Cr 22.5; Mo 9.5	Ni	0.07
Nickel-based alloy with 1.5% Be	Ni 76; Cr 13; Mo 3; Be 1.5	Ni	101
		Cr	5.6
		Mo	2.3
		Be	10.3
CPTi (grade 2, cast)	Ti 99	Ti	0.36
NPG	Cu 79.3; Al 17.8; Ni 4.3; Fe 4; Zn 3; Mn 1.6	Cu	1226
		Al	117
		Ni	67
		Fe	45
		Zn	40
		Mn	32

From Kappert *et al.* (1998)
CPTi, commercially pure Ti; NPG, non-precious gold

A similar analysis of various types of alloy used in orthodontics has shown that nickel and chromium were released when these alloys were stored in physiological saline. Soldered stainless steel bows were very susceptible to corrosion. The release of nickel seemed to be related to both the composition of the alloys and the method of manufacture of the appliances, but was not proportional to the nickel content (Grimsdottir *et al.*, 1992).

5A.2 Absorption, distribution and excretion

Implants in soft tissue or bone give rise to exposure in that tissue, and the biological effects depend on the interactions of the tissue with the surface of the implant. Such

effects may remain localized to the site of the implant itself, but metal ions or particulate debris released as a result of corrosion or wear may cause effects at distant sites. Oral exposures prevail in the case of dental fillings, while materials released from these may be ingested and give rise to exposure via the gastrointestinal tract.

Published studies of biological effects of implants in surrounding tissue have various limitations; many were based on cases of failed implants, which may have led to additional corrosion and/or wear, hence to stronger effects than would normally be the case. Furthermore, the composition and properties of the implants are often poorly described. In addition, it is not always clear whether the effects observed are due to the implant itself or to material released from it. In terms of their possible carcinogenic hazard, metal ions released systemically can be regarded as metal salts, several of which have been the subject of previous IARC Monographs (IARC, 1990a,b, 1991, 1993a,b). When metal ions are released systemically, accumulation may occur in specific organs. Thus nickel accumulates in the liver, spleen and kidney of mice after administration of high doses of the metal ion (Pereira *et al*., 1998), vanadium accumulates in the liver, spleen and bone, but titanium is reported to accumulate less (Merritt & Brown, 1995). A complex mixture of ionic species may be formed during corrosion, and a wide range of sizes, shapes and numbers of particles may be produced by wear, but many reports provide very little information on these aspects.

5A.2.1 *Humans*

Doorn *et al*. (1998) described the analysis of metallic particles in tissue from 13 patients with cobalt–chromium–molybdenum metal-on-metal total hip implants for periods ranging from seven months to 25 years. Samples were obtained at either revision or autopsy from different sites around the implant and particles were found in eight patients. There was marked inter- and intra-individual variability in both the number (range, 1–580) and size (range, 6–834 nm, most < 50 nm) of particles. The authors had previously determined volumetric wear in three of these patients (McKellop *et al*., 1996). They estimated that wear produced 6.7×10^{12}, 4.9×10^{13} and 2.5×10^{14} particles per year in three metal-on-metal prostheses and contrasted these figures with their estimate for polyethylene wear debris of 5×10^{11} particles per year in a metal-on-polyethylene hip implant.

Willert *et al*. (1996) determined metal concentrations in tissue samples from 19 patients with cobalt–chromium metal-on-metal total hip implants for periods of one to 282 months (average, 86). Samples were obtained at revision from various sites around the implant, mainly the joint capsule, and metal particles were found in 15 patients. As far as they were visible in the light microscope, these were irregularly shaped, ranged from 0.5 to 5 μm in size and were most often found in the vicinity of blood vessels. Poly(methyl methacrylate) bone cement particles were found at greater concentrations than metal particles. Both the worn surface and the wear particles undergo repassivation to cobalt(II) hydroxide, chromium(III) oxide, chromium(III) hydroxide and nickel(II) hydroxide. While these cobalt and nickel compounds have

solubilities greater than 10^{-4} M at physiological pH, the chromium compounds are essentially insoluble under the same conditions, so that chromium accumulates in local tissues while the other products are eliminated by urinary and faecal excretion. Merritt and Brown (1996) reported that the estimated dissolution of cobalt–chromium alloy is 0.15–0.30 μg/cm^2 per day, which corresponds to around 11 mg per year for a total hip replacement. This can be increased by corrosion.

Jacobs *et al*. (1996) determined cobalt and chromium concentrations in serum and chromium concentrations in urine from eight patients with long-term (> 20 years) cobalt–chromium metal-on-metal total hip replacements, six patients with short-term (< 2 years) cobalt–chromium metal-on-metal surface replacement arthroplasties and three controls. Single samples were obtained at average implantation times of 295 (range 266–324) months from the total hip replacements and 12.4 (range 2–19) months from the surface replacements. No details of diet were recorded, nor was this variable controlled. The controls had chromium concentrations in serum and urine of 0.14 ng/mL (2.7 nmol/L) and 0.035 ng/mL (0.66 nmol/L), respectively, and cobalt concentrations in serum were below the detection limit (0.3 ng/mL, 5.2 nmol/L). The mean total 24-h urinary chromium excretion in the controls was 0.071 μg per day (1.37 nmol per day). The mean serum chromium and cobalt concentrations in the total hip replacement patients were nine- and threefold higher respectively, whilst urinary chromium concentrations were 35-fold higher than in the controls. The mean total 24 h urinary chromium excretion was 30-fold higher than in the controls. The mean serum chromium and cobalt concentrations in the subjects with surface replacements were three- and four-fold higher, respectively than in the hip replacement patients, whilst urinary chromium concentrations were four-fold higher. The mean total 24-h urinary chromium excretion was 2.5-fold higher in the surface replacement group than in the hip replacement subjects.

In a study to investigate nickel and chromium concentrations in saliva of patients with different types of fixed dental appliances (containing 8–12% nickel and 17–22% chromium), fresh saliva samples were obtained from each of 47 orthodontic patients before insertion of the appliance and 1–2 days, 1 week and 1 month after treatment. The method of sampling shows the momentary total concentration of soluble nickel and chromium. The saliva concentrations of both metals showed considerable variation, and no significant differences were found in samples taken before and after treatment. The authors note that minor amounts of nickel released from dental fixtures could be important in case of hypersensitivity to nickel or in evoking allergic reactions in the oral mucosa (Kerusuo *et al.*, 1997).

5A.2.2 *Experimental systems*

Harmand *et al*. (1994) described the dissolution in culture medium and cellular uptake in an osteoblast cell line of ISO 5832/3 titanium alloy, ISO 5832/1 316L stainless steel and ISO 5832/4 cobalt–chromium alloy (defined in Tables 8–10) over a nine-day period. The presence of the cells had varying effects (increase, decrease or none) on release of metal ions from these metals. Uptake of extracellular ions by the

cells was limited to chromium, vanadium, titanium, iron and cobalt, with the highest uptake been observed for chromium.

Gray and Stirling (1950) exposed serum and red blood cell cultures to radioactive chromium (^{51}Cr) with a valency of +3 ($^{51}CrCl_3$) and with a valency of +6 ($Na_2{}^{51}CrO_4$). Almost all of the trivalent chromium remained in the plasma, whereas hexavalent chromium crossed the red cell membrane and was primarily cell-associated. Similar results were obtained with fretting corrosion experiments in cell culture (Merritt *et al*., 1991). These results imply that the valency of chromium affects its biological activity. It is clear that the biological fate of corrosion products needs to be understood before conclusions can be drawn regarding the relevance of chemical analytical data of tissues and fluids for effects of implant corrosion.

In a study to investigate the effect of anodization on the dissolution of titanium, Sprague-Dawley rats were given anodized or unanodized titanium implants intraperitoneally, in the left paracolic gutter. At days 7, 14 and 28, peritoneal lavages and blood samples were obtained. At day 28 the animals were killed and liver, kidneys, spleen, lung and brain were removed, as well as tissue surrounding the implant. Titanium was not detected in any distant organs or in the lavage fluid. In the capsular tissues surrounding the implants titanium concentrations were higher in animals with unanodized implants than in those with anodized implants, but the difference was not significant. Peritoneal leukocytes showed significantly higher titanium levels in animals from the unanodized implant group, compared with the controls, while titanium levels in leukocytes from animals with anodized implants were not significantly different from the controls. Despite the presence of titanium in leukocytes, only minimal biological responses and histopathological changes were detected. The presence of titanium in the tissue surrounding the implants is probably the result of corrosion. Surface treatment of titanium by anodization reduces passive dissolution (Jorgenson *et al*., 1999).

To examine the biological transport of released metal ions, Merritt *et al*. (1984a) injected metal salts (nickel chloride, cobalt chloride, chromium chloride, potassium dichromate) intramuscularly into hamsters. Blood samples were taken at 2, 4, 6, 24, 48 and 96 h after injection. Nickel was found in the blood serum at 2, 4 and 6 h, but the levels dropped rapidly. Levels of nickel in red and white blood cells were low. Cobalt and trivalent chromium were similarly found in serum, but the levels did not drop as rapidly. In contrast, hexavalent chromium from potassium dichromate was found in the red blood cells, confirming the results of Gray and Stirling (1950). Corrosion products generated by fretting corrosion of 316 LVM stainless steel or MP35N plates and screws were suspended in serum and injected intramuscularly into hamsters; chromium was again found in the red blood cells. When serum that had interacted with the metal salts or corrosion products was separated into its components by isoelectric focusing on polyacrylamide gels, almost all of the metal, whatever the source, was detected in the albumin region of the gels, indicating strong albumin binding (Merritt *et al.*, 1984b).

Brown *et al*. (1988) carried out chemical analysis of urine from Syrian hamsters after intramuscular injection of nickel(ous) chloride, cobalt chloride and potassium dichromate, or after accelerated anodic corrosion both *in vitro* and *in vivo* of stainless steel implants. The amounts of metal injected were 90 µg of nickel, 94 µg of cobalt and 117 µg of chromium in one group of animals, and 5.18 µg of nickel, 5.40 µg of cobalt and 6.91 µg of chromium in the second group. Total daily urine samples were collected during three days. In both dose groups, virtually all of the injected nickel and most of the cobalt were excreted in the first 24 h, whereas less than 50% of the chromium dose was excreted. After accelerated anodic corrosion of stainless steel, nickel excretion was complete within 24 h, while chromium excretion was minimal. Similar studies were performed with rods of a nickel–cobalt–chromium–molybdenum alloy (F 75) with a porous coating. These rods were implanted subcutaneously in Syrian hamsters and subjected to accelerated anodic corrosion *in situ*. Even though the nickel content of F 75 alloy is less than 1%, it was rapidly excreted and detected in the urine, as was the molybdenum. Recovery in urine of cobalt was close to 80%, whereas that of chromium was in the range of 37–67% due to *in vivo* storage and significant binding of chromium to red cells (Brown *et al*., 1993).

These studies also showed that the corrosion rates of these alloys in 10% serum were much lower those that in saline. The rates *in vivo* were similar to those in serum. Thus, for testing materials for corrosion, the use of proteins in the test solution provides a better simulation of the in-vivo environment (Brown *et al*., 1988).

The implication of these results is that chemical analysis of body fluids and tissues must be interpreted in light of the mechanism of degradation. If an implant corrodes and releases metal ions, nickel and cobalt will be transported and excreted, while chromium may be cell-bound, either in local tissues or in organs such as the lung, kidney, liver and spleen. Thus tissue levels may be different from that of the alloy composition. Also, if there is significant wear and particulate debris in the tissues, chemical analysis of the tissue will indicate a composition different from that of the alloy.

5A.3 Tissue responses and other expressions of toxicity

5A.3.1 *Humans*

(*a*) *Inflammatory and immunological responses*

No relevant systematic studies of tissue responses in orthopaedic implant patients have been reported, although some reviews of case reports have tried to link the occurrence of tumours to carcinogenic mechanisms. In a review of nine cases of implantation site tumours following knee arthroplasty, and in a wider-ranging review of the use of metallic implants, it was suggested that carcinogenicity could result from the release of carcinogenic corrosion products (Jacobs & Oloff, 1985; Wapner, 1991).

A case series of 20 failed hip replacements (two Charnley (metal (TiAlV)-on-polyethylene) and 18 McKee-Farrar (metal-on-metal)) revealed mild to severe acute inflammatory response (characterized by the predominant occurrence of polymorpho-

nuclear leukocytes) in all 12 cases that failed due to infection. The remaining eight cases failed due to loosening. Chronic inflammation was seen in all but one of the 12 infected cases (predominantly lymphocytes and plasma cells). Acrylic debris from the cement was found in all cases extracellularly and in three also intracellularly; one patient from this group and one other patient showed polyethylene debris (Charosky *et al*., 1973).

A case was reported of aseptic aggressive granulomatosis seven years after knee arthroplasty. Both titanium- and polyethylene-containing fragments were observed around the prosthesis, some titanium being found within macrophages or giant cells (Tigges *et al*., 1994).

None of the pathological changes noted in clinical reports are suggestive of pre-cancerous states.

When a material is implanted, it is recognized as a foreign body and macrophages adhere to the surface of the material (Tang *et al.*, 1993; Mrksich & Whitesides, 1996). Large amounts of macrophages and polyethylene debris are observed in tissues around aseptically loosened hip arthroplasty (Dorr *et al*., 1990; Wroblewski, 1997). Macrophages generate active oxygen species by themselves without being active in phagocytosis, but the production of active oxygen is much higher during this process (Johnston & Kitagawa, 1985; Edwards *et al*., 1988). The primary oxygen radical (O_2^{-}) is converted by superoxide dismutase to hydrogen peroxide, which penetrates the metal surface to which the macrophage has adhered. In the case of a titanium implant, hydrogen peroxide reacts with the surface oxide film of titanium, which results in the formation of a stable $TiOOH(H_2O)_n$ complex. This TiOOH matrix traps the superoxide radical so that no or very small amounts of free hydroxyl radicals are formed. Apart from titanium, other biocompatible metals such as zirconium and aluminium also show low hydroxyl radical production (Tengvall *et al*., 1989).

(*b*) *Oral contact lichenoid reactions*

Contact lichenoid reactions topographically related to dental restorations display various clinical characteristics, ranging from asymptomatic papular, reticular and plaque type lesions to symptomatic atrophic and reticular lesions (Holmstrup, 1991). Contact lesions present with similar clinical characteristics as oral lichen planus. These two types of lesion can be discriminated only by the degree to which the oral mucosa is involved (Bolewska *et al*., 1990a,b). By definition, contact lesions are limited to areas of frequent contact with dental restorations, whereas oral lichen planus also involves other regions of the oral mucosa. In a study of the effect of replacement of dental amalgam with gold or metal–ceramic crowns on oral lichenoid reactions, Bratel *et al*. (1996) found that the lesions showed considerable improvement in 95% of the patients. This effect was parallelled by a disappearance of symptoms, in contrast to patients with persisting contact lesions (5%), who did not report any significant improvement. The healing response was not found to correlate with age, gender, smoking habits, subjective dryness of the mouth or current medication. The healing

effect in patients who received gold crowns was superior compared with that of patients receiving metal–ceramic crowns. Similar contact lesions have been seen in the topographical relationship to dental composite restorations (Lind, 1988) and palladium-based crowns (Downey, 1989).

The etiology of these lesions remains uncertain. An immunological mechanism is involved in some cases, whereas others seem to be related to irritative or cumulative insult-type reactions. Microbial factors such as viral or fungal infections may also contribute to the clinical appearance. It is unclear whether oral lichen planus is a multivariant group of etiologically diverse diseases or a disease entity characterized by a type IV hypersensitivity reaction to an antigen in the junction zone between epithelium and connective tissue. The premalignant potential of oral lichenoid lesions requires regular follow-up at three- to six-month intervals (for reviews, see Scully *et al.*, 1998; Holmstrup, 1999).

(*c*) *Allergic reactions*

In questionnaire surveys about side-effects associated with dental materials, the prevalence was estimated to be 1:300 in periodontics and 1:2600 in pedodontics. None of these reactions was related to dental casting alloys. In prosthodontics, the prevalence was calculated to be about 1:400, and about 27% were related to base-metal alloys for removable partial dentures (cobalt, chromium, nickel) and to precious-metal-based alloys for porcelain-fused-to-metal restorations. The complaints consisted of intra-oral reactions (such as redness, swelling and pain of the oral mucosa and lips), oral/gingival lichenoid reactions and a few instances of systemic allergic reactions. In orthodontics, the prevalence was 1:100, and most reactions (85%) were related to metal parts of the extra-oral anchorage devices (Hensten-Pettersen, 1992).

Even though the extensive use of base-metal alloys has been of major concern to the dental profession, relatively few case reports of allergic reactions substantiate this concern. Allergy to gold-based dental restorations has been more commonly reported. Palladium-based alloys have been associated with several cases of stomatitis and oral lichenoid reactions. Palladium allergy seems to occur mainly in patients who are highly sensitive to nickel. All casting alloys, except titanium, seem to have a potential for eliciting adverse reactions in individual hypersensitive patients. Induction of tolerance may be a possible benefit of the use of intra-orally placed alloys. In non-sensitized individuals, oral antigenic contacts to nickel and chromium may induce tolerance rather than sensitization (Hensten-Pettersen, 1992).

Both local and systemic reactions may sometimes occur following implantation of metallic devices (Rostoker *et al.*, 1986; Wilkinson, 1989; Guyuron & Lasa, 1992). Metal allergy has been suggested as a predisposing factor for infection of periprosthetic tissues (Hierholzer & Hierholzer, 1984). However, the majority of individuals—even the majority of sensitized individuals—seem to tolerate low levels of allergens in the tissues without adverse effect. Induction of immunological tolerance may be a potential benefit.

The mechanisms by which local cutaneous and systemic reactions are induced by nickel in orthopaedic implants remain obscure and the development of such reactions is unpredictable. Some reactions appear to be type I in nature. In others, there is good evidence of type IV hypersensitivity. In some patients, however, type I, III (Arthus) and IV reactions seem to coexist (Wilkinson, 1989). The reaction patterns elicited by other metals seem to be similar to those induced by nickel.

5A.3.2 *Experimental systems*

(*a*) *Animal studies*

The chemical carcinogenicity of metal compounds (such as that of chromium, nickel, cobalt and arsenic) is believed to be dependent largely on their oxidation state and solubility, with oxidative DNA damage or interference with DNA repair having been postulated as likely mechanisms (Hartwig *et al.*, 1996). Additional mechanisms of metal carcinogenesis include epigenetic changes, chromatin condensation or altered patterns of gene methylation (Costa, 1997; Salnikow *et al*., 1997). However, very few experimental studies have provided information on tissue responses to metallic implants that is of relevance to carcinogenicity.

A series of five 18–24-month studies of tumour incidence following implantation of tin revealed unusual non-neoplastic pathology, but only in tumour-bearing groups. The studies were carried out in female Marsh mice and male and female Evans rats given intraperitoneal tin implants (open-end cylinders, 12×4 mm in mice, 25×8 mm in rats). In addition to tumours (an increase was seen in local sarcomas with metastases, but not in spontaneous lymphoid tumours in rats), atypical mesothelial hyperplasia, adenomatous hyperplasia and osseous metaplasia were noted. Chronic inflammatory responses were also common. These included focal histiocytic aggregation, multinucleate giant cells, granulomata, fibrohyalinized capsular tissue, necrosis, fibrocellular fat, lack of capsule, hyalinized sclerosis and cysts (Bischoff & Bryson, 1977).

Male WAB rats, 20 weeks of age, were given implants of either Walker 256 carcinoma cells (as 5-mg solid tumour fragments) or syngeneic neonatal thymus tissue. These tissues were placed in the centre of platinum–silicone elastomer loops, which had been implanted earlier. Control animals received tissues without implants. Colchicine was used to facilitate the assessment of cell proliferation. Rats with thymus grafts were killed after three weeks and those with tumour grafts after up to six days. Group sizes were not reported. Historical data indicated 100% tumour growth at six days in 1500 controls, whereas tumour growth was inhibited in 200/230 rats with implants. Marked reductions in cell density and mitotic rate were seen, in comparison with controls, at two, three, four and six days after tumour implantation, the cell density being lowest close to the implant. Unlike in controls, proliferation of tumour cells in the vicinity of implants was concentrated into foci of intense activity. In contrast, proliferation of thymus tissue was unaffected by the presence of the platinum–silicone implant. The authors ruled out restriction of the blood supply as a reason for the observed effects and suggested that the selective inhibition of tumour cell proliferation

may be a result of an alteration of the electrochemical environment by the implant (Hinsull *et al*., 1979). [The Working Group found it difficult to interpret these data.]

(*b*) *Cytotoxicity of metal ions*

The cytotoxicity of metal ions has been investigated systematically in L-929 fibroblasts (Takeda *et al*., 1989; Schedle *et al*., 1995; Yamamoto *et al.,* 1998) and 3T3 fibroblasts (Wataha *et al*., 1991; Yamamoto *et al*., 1998). The rank orders of cytotoxicity that were found are: Cr > Co > V > Fe > Mn > Cu > Ni >> Mo (Takeda *et al*., 1989); $Cd^{2+} > Ag^{+} > Zn^{2+} > Cu^{2+} > Ga^{3+} > Au^{3+} > Ni^{2+} > Pd^{2+} > In^{3+}$ (Wataha *et al*., 1991), $Ag^{+} > Pt^{4+} > Co^{2+} > In^{3+} > Ga^{3+} > Au^{3+} > Cu^{2+} > Ni^{2+} > Zn^{2+} > Pd^{2+} > Mo^{5+} > Sn^{2+} > Cr^{2+}$ (Schedle *et al*., 1995); $Cd^{2+} > In^{3+} > V^{3+} > Be^{2+} > Sb^{3+} > Ag^{+} > Hg^{2+} > Cr^{6+} > Co^{2+} > Bi^{3+} > Ir^{4+} > Cr^{3+} > Hg^{+} > Cu^{2+} > Rh^{3+} > Tl^{3+} > Sn^{2+} > Ga^{3+} > Pb^{2+} > Cu^{+} > Mn^{2+} > Tl^{+} > Ni^{2+} > Zn^{2+} > Y^{3+} > W^{6+} > Fe^{3+} > Pd^{2+} > Fe^{2+} > Ti^{4+} > Hf^{4+} > Ru^{3+} > Sr^{2+} > Sn^{4+} > Ba^{2+} > Cs^{+} > Nb^{5+} > Ta^{5+} > Zr^{4+} > Al^{3+} > Mo^{5+} > Rb^{+} > Li^{+}$ (data for 3T3 cells; Yamamoto *et al*., 1998). The concentrations that reduced [^{3}H]thymidine incorporation to 50% of the control ranged between 0.4 and > 435 μmol/L (Wataha *et al*., 1991) and between 0.017 mmol/L and > 1 mmol/L (Schedle *et al*., 1995). Yamamoto *et al.* (1998) calculated the IC_{50} values (50% of cell growth inhibition), which ranged from 1.36×10^{-6} to 1.42×10^{-2} (mol L^{-1}). For both cell types studied, the ions of chromium, cadmium, vanadium, silver and cobalt were generally the more cytotoxic. Sun *et al.* (1997) tested the effects of Al^{3+}, Co^{2+}, Cr^{3+}, Ni^{2+}, Ti^{4+} and V^{3+} on osteoblast-like cell metabolism and differentiation. DNA synthesis, succinate dehydrogenase and alkaline phosphatase activities, culture calcification and osteocalcin and osteopontin gene expression were investigated in ROS 17/2.8 cells. It was shown that metal ions can alter osteoblast behaviour at subtoxic concentrations, but do not affect the expression of all genes similarly. Granchi *et al*. (1998) showed that large amounts of nickel and cobalt extracted from the metal powders induced necrosis *in vitro* in mononuclear cells from human peripheral blood and high concentrations of chromium or limited amounts of nickel and cobalt caused cell death by apoptosis. The cytotoxicity of metal ions extracted from commercial gold alloys, silver alloys and nickel–chromium alloys was tested on L929 mouse fibroblasts (Schmalz *et al*., 1998a). The TC_{50} values were slightly lower in corresponding salt solutions than in extracts. Nickel and cobalt ions upregulated the expression of adhesion molecules as well as of the cytokines interleukin (IL)-6 and -8 in human endothelial cell cultures, as do proinflammatory mediators (Wagner *et al.*, 1998). Silver, mercury and, to a lesser extent, gold ions induced direct toxicity (histamine release, ultrastructural signs of necrosis) and platinum ions induced cell death through induction of apoptosis in the human mast cell line HMC-1 (Schedle *et al*., 1998a). Extracts from titanium–nickel alloy (50:50) were not cytotoxic *in vitro*, not allergenic *in vivo* in guinea-pig, nor genotoxic *in vitro* in *Salmonella typhimurium* for gene mutation or in V79 cells for chromosomal aberrations (Wever *et al*., 1997). Extracts from cobalt–chromium orthopaedic alloys caused inhibitory effects on cell viability, on alkaline phosphatase activity and, to a lower extent, on protein production in all rat, rabbit and human bone-marrow

cell cultures tested, the human cells being most sensitive to exposure to metal ions (Tomás *et al.*, 1997). Ions associated with the titanium–chromium–vanadium alloy Ti 6,4 inhibited the normal differentiation of rat bone-marrow stromal cells to mature osteoblasts *in vitro* (Thompson & Puleo, 1996).

(*c*) *Cytotoxicity of metallic materials*

Cobalt–chromium alloy was toxic to macrophages *in vitro*, as reflected by release of tumour necrosis factor (TNF) α, prostaglandin E2 and the enzyme lactate dehydrogenase (Horowitz *et al.*, 1998). Test specimens fabricated from copper, cobalt, zinc, indium, nickel and precious-metal cast alloys reduced cell viability by 10–80% (copper being the most active) in a three-dimensional cell-culture system consisting of human fibroblasts and keratinocytes (Schmalz *et al.*, 1998b). Nickel–titanium (Nitinol) did not induce cytotoxic effects in human osteoblasts and fibroblasts *in vitro* (Ryhänen *et al.*, 1997).

(*d*) *Effects of metal ions and metallic materials on cytokine levels and histamine release*

Effects of dental amalgam and heavy metal cations on cytokine production by peripheral blood mononuclear cells were investigated *in vitro* (Schedle *et al.*, 1998b). Fresh amalgam specimens and salt solutions containing Cu^{2+} and Hg^{2+} induced a decrease in interferon-γ and IL-10 levels, whereas fresh amalgam specimens and Hg^{2+} caused an increase in TNF-α levels. Amalgam specimens preincubated in cell-culture medium for six weeks did not cause any effects. Ag^{+}, Au^{3+} and Hg^{2+} induced rapid histamine release from human tissue mast cells *in vitro* (Schedle *et al.*, 1998a). Exposure of macrophages cocultured with osteoblasts to cobalt–chromium alloy led to significant release of TNF-α and prostaglandin E2, but no significant IL-6 or IL-1β production (Horowitz *et al.*, 1998).

5A.4 Genetic and related effects

5A.4.1 *Humans*

Case *et al.* (1996) studied chromosomal aberrations in blood and bone marrow in 71 patients (mean age 73 years) with hip (n = 69) or knee (n = 2) replacements who required revision surgery because of worn prostheses, and in 30 control patients (mean age 70.3 years) having primary arthroplasty. Bone marrow was taken at the site of the worn prostheses in the case of revision surgery or at the site of the newly inserted prostheses in the case of primary arthroplasty. Bone marrow from the ipsilateral iliac crest and peripheral blood samples were also taken from all patients for metaphase analysis. The frequency of chromosomal aberrations (mean ± SD) in marrow taken from the femur at primary arthroplasty (5.8 ± 4.3 aberrant cells per 100 cells) was not different from that found in the iliac crest marrow taken from revision cases (4.6 ± 3.3 aberrant cells per 100 cells). However, there was a significant increase in the frequency of chromosomal aberrations (mean ± SD; aberrant cells per 100 cells) in the cells taken from

the femoral marrow adjacent to a worn prosthesis (11.4 ± 10.2, $n = 16$, ≤ 10 years after primary arthroplasty; 12.7 ± 10.8, $n = 9$, > 10 years) compared with the frequency in the iliac crest marrow from the same patients (3.3 ± 2.7, $n = 11$, ≤ 10 years; 5.6 ± 2.0, $n = 10$, > 10 years) or with femoral marrow from patients at primary arthroplasty (see above). Nine out of 27 femoral marrow samples from revision cases had higher chromosomal aberration frequencies (17–40 aberrations/100 cells) than any of the control femoral bone marrow (1–15 aberrations/100 cells) or iliac crest marrow samples from revision cases (0–15 aberrations/100 cells). Chromosomal aberration frequencies were only slightly higher in patients requiring revision more than 10 years after primary arthroplasty. Two patients with a long duration of arthroplasty (18 and 20 years) showed clonal expansion of B or T cells which was associated in one case with a high level of chromosomal aberrations in the femur (26/100 cells) compared with ipsilateral iliac crest (6/100 cells). The authors cautioned that the results should be seen as preliminary due to the low patient numbers. They discounted concomitant disease and X-rays as predisposing factors, leaving wear debris as a potential causative agent.

5A.4.2 *Experimental systems*

Very few mutagenicity studies have been performed with metallic medical and dental materials. The evaluation of the mutagenic potential is normally based on either the results of tests with extracts or on knowledge of the mutagenic potential of the individual metallic components of the biomaterial.

(*a*) *Genotoxic activity of metals and metal compounds*

Data on the genotoxicity and mutagenicity of some of the metals used in implants have been compiled in previous IARC Monographs and are summarized here.

Chromium[VI] compounds of various solubilities in water were consistently active in numerous studies covering a wide range of tests for genetic and related effects (IARC, 1990a).

The chromium[III] compounds tested were generally not genotoxic in numerous studies and only weak effects were observed in some tests (IARC, 1990a).

Soluble nickel compounds were generally active in the human and animal cells in which they were tested *in vitro* (IARC, 1990b).

Cobalt[II] compounds induced DNA damage, mutations, sister chromatid exchanges and aneuploidy in mammalian cells. Some cobalt[III] complexes with heterocyclic ligands were also active in these assays (IARC, 1991).

Chromosomal aberrations and aneuploidy were observed in mammalian cells *in vitro* and in rodents *in vivo* after treatment with cadmium chloride. DNA strand breaks, mutations, chromosomal damage and cell transformation have been observed after exposure of mammalian cells to cadmium compounds *in vitro* (IARC, 1993b).

DNA damage, sister chromatid exchanges, chromosomal aberrations and aneuploidy (spindle disturbances) have been induced by mercury compounds in mammalian

cells *in vitro*. Weak positive genotoxic effects were observed with mercuric chloride in rodents *in vivo* (IARC, 1993a).

Beryllium salts are not mutagenic in most bacterial systems but they induced sister chromatid exchanges and possible chromosomal aberrations in mammalian cells *in vitro*. Beryllium chloride induced gene mutation and chromosomal aberrations in mammalian cells *in vitro* (IARC, 1993c).

(*b*) *Tests using extracts*

Extracts for testing are generally prepared by adding the biomaterial to water, saline or cell-culture medium for a few hours or days at 37°C. The corrosion that occurs under these conditions has generally not been compared with the corrosion that is observed *in vivo*. Also, an analysis of leachable material is often not made, so there is no assurance that any such substances have indeed been extracted from the biomaterial. Reference to the mutagenicity of individual metallic components can be misleading, because the ionic species tested may be different from those generated by leaching.

Tests on extracts are often performed for regulatory compliance, and results are not usually published in the open literature. [The Working Group noted that many genotoxicity tests carried out for such purposes are not adequate to identify all mutagenic hazards and they may not address all relevant mutagenic end-points or optimize exposure to the test system.]

Assad *et al*. (1998) studied single-strand DNA breakage in interphase and metaphase human lymphocytes *in vitro* using an in-situ end-labelling method with electron microscopy. Twenty-four-hour extracts of particles of a nickel–titanium alloy (diameter 250–500 μm) were prepared using complete RPMI medium at 37°C. The lymphocytes were exposed to the extracts for 72 h. The results were compared with those obtained with extracts of commercially pure titanium or 316 L stainless steel particles. No determination of the quantity of each metal extracted by the medium was performed. DNA strand breaks were significantly increased in metaphase chromatin with extracts of 316 L stainless steel. Extracts of the nickel–titanium alloy or of pure titanium did not show an effect. However, no information on the metallic species from stainless steel producing the effect was provided. No effect on chromatin in interphase nuclei was observed with any of the extracts.

Wever *et al*. (1997) tested a nickel–titanium alloy (50% nickel) and compared the results with those obtained with stainless steel containing 13–15% nickel. Both alloys were extracted in aqueous 0.9% sodium chloride for 72 h at 37°C. No determination of the metal content of the extracts was performed. Extracts were tested with and without metabolic activation for mutagenic activity in four strains of *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and for induction of chromosomal aberrations in Chinese hamster ovary (CHO) cells. The two alloys gave negative results in both tests.

The potential to induce neoplastic transformation in C3H T½ fibroblasts was tested for eight metals (cobalt, chromium, nickel, iron, molybdenum, aluminium, vanadium and titanium) and their alloys (stainless steel, chromium alloy, titanium–aluminium–

vanadium alloy). The cells were exposed to solutions of the metal salts or to metal or alloy particles (particle size ≤ 5 μm). Cell transformation was observed with soluble forms of cobalt, chromium, nickel and molybdenum, although in some cases only at cytotoxic concentrations. Vanadium, iron, aluminium and titanium salts did not induce cell transformation. The particulate metals and alloys failed to induce cell transformation, although large differences in toxicity were noted (Doran *et al*., 1998).

5A.5 Mechanisms of carcinogenic action

Surgical alloys and metallic medical devices are insoluble in physiological media, but are subject to corrosion and wear. Corrosion can result in the release of soluble ions. Information with respect to the potential carcinogenic hazard of such ions is available in previous IARC Monographs. However, it is difficult to evaluate whether the metal salts play any significant role in the possible carcinogenic effects observed with implants, because there is a striking lack of data on the actual amounts of ions released into the surrounding tissue and on the nature of the ionic species involved. It is possible that irritation of the surrounding tissue by the implant itself, presumably occurring at the boundary between the metal surface and the tissue, can provoke responses that lead to disturbances of normal cellular function. In addition, inflammatory reactions observed at the site of implants may enhance oxidative processes, inducing cellular damage and regenerative cell proliferation. Particulate debris generated by wear may induce similar reactions in the surrounding tissue or at more distant sites. One single study has reported an enhanced frequency of chromosomal aberrations in cells adjacent to a loose or worn prosthesis (hip or knee replacement) in elderly patients (Case *et al.*, 1996).

5B. Non-metallic Medical and Dental Materials

Of the many components of non-metallic medical and dental materials, only a few are discussed here, which are those for which some data are available.

5B.1 Degradation, distribution, metabolism and excretion

5B.1.1 *Humans*

(*a*) *Degradation of polyurethane foam*

Following early studies on degradation of polyurethane foam in implants in humans, it was widely suggested that the foam either broke up or disappeared. However, Szycher and Siciliano (1991) considered that the apparent fragmentation was observed because of ingrowth of tissue into the foam structure and preparation of histological sections cut through the three-dimensional matrix of the foam. It is however clear that degradation of the foam *in vivo* can lead to loss of at least 30% over nine years.

The urine of a female patient was analysed following implantation of Même® polyurethane-covered breast implants. The implants were replaced at a revision operation

during which partial absorption of the foam was observed and scar tissue was removed. Urine, collected at several times over a three-month period after implantation, was analysed by gas chromatography/mass spectrometry (GC/MS) of heptafluorobutyryl derivatives following extraction with toluene and cleavage of acetyl conjugates of 2,4-diaminotoluene in urine by boiling in 6 M hydrochloric acid for 1 h. Scar tissue was extracted and hydrolysed in a similar manner. No free 2,4- or 2,6-diaminotoluene was detected in either urine or scar tissue. In acid-hydrolysed scar tissue, total 2,4-diaminotoluene levels were 27.22 μg/g and total 2,6-diaminotoluene levels were 6.02 μg/g. In acid-hydrolysed urine, total 2,4-diaminotoluene levels ranged from 0.27 to 1.69 μg/L and total 2,6-diaminotoluene from 0.11 to 0.61 μg/L. These levels appeared to be related to creatinine clearance values. The authors recognized that the acid hydrolysis of urine could potentially cleave polyurethane oligomers as well as the expected acetyldiaminotoluene metabolites, but doubted whether such oligomers would be readily absorbed and circulated. They suggested that cleavage to toluene diisocyanates with subsequent conversion to diaminotoluenes and acetylation of diaminotoluenes was more likely to occur. In the case of oligomer cleavage, the urinary ratio of 2,4-diaminotoluene to 2,6-diaminotoluene should be the same as in the polymer i.e., 4:1, but would not necessarily be so in the event of in-situ breakdown, due to possible steric effects at enzyme active sites. The observed ratio was 2.4:1, which supports the acetylaminotoluene pathway (Chan *et al*., 1991a).

The urine of a second female patient was analysed following implantation of Même® polyurethane-covered breast implants. Urine was collected at several times over a seven-month period after implantation and a control sample was obtained before implantation. Extraction, conjugate cleavage and analysis were performed as described above; however, the method was modified by addition of methylenediphenyldiamine as an internal standard before extraction. The first sample, collected 21 days after implantation, and all subsequent samples collected over a seven-month period, contained both free and acid-hydrolysable 2,4-diaminotoluene and 2,6-diaminotoluene. The free 2,4- and 2,6-diaminotoluene levels were 0.47 to 0.92 μg/L and 0.13 to 0.34 μg/L, respectively, equivalent to 4.8–9.0% and 3.1–6.5% of the total, respectively. The total 2,4- and 2,6-diaminotoluene levels were 5.2–18.1 μg/L and 2.0–8.5 μg/L, respectively. The levels appeared to be related to creatinine clearance values. The concentrations in urine were relatively consistent at all time points, possibly reflecting steady-state kinetics. The acetyl metabolite was not measured directly, but the ratio of 2,4-diaminotoluene to 2,6-diaminotoluene was again less than in the polymer (Chan *et al.,* 1991b).

The fibrous capsule and polyurethane foam recovered following explantation of breast implants were studied to characterize the biodegradation of polyurethane foam *in vivo*. Seventy-five freshly retrieved polyurethane-coated breast implants and surrounding capsule from 47 patients were analysed. The reasons for removal were capsular contracture (48%), infection or exposure of prosthesis (13%) or other (39%). Tissue from several sites around the surface of the implants was digested in a collagenase solution until either foam was recovered or all the tissue was digested. Additional samples were fixed in 10% formalin and were examined histologically. Visibly intact foam was

recovered from only 48% of the prostheses after enzymatic digestion of capsular tissue. There was a progressive decline in the recovery of visibly intact foam with increasing time since implantation. Scanning electron microscopy showed fractures and fissures in the foam structure and thinning of the polyurethane struts from 49 ± 1.5 μm in control (unimplanted) foam to 30 ± 3.1, 32 ± 3.1 and 41.2 ± 2.3 μm in those removed due to contracture, infection or other reasons, respectively. In all but two of the samples without visibly intact foam, residual polyurethane foam was observed in the capsule by light microscopy. Histological examination revealed scalloping and fracturing of the foam. Overall, there was convincing evidence that the foam had degraded *in vivo*. It was not possible to quantify the rate of degradation accurately, but capsular contracture, infection and time appeared to have an effect on polyurethane biodegradation in the human body (Sinclair *et al.,* 1993).

A case–control study was conducted among 129 women, of whom 66 had polyurethane foam-coated breast implants and 63 were age- and race-matched controls with an age range of 23 to 62 years. Women who had known contact with either polyurethane such as contraceptive sponges or other polyurethane-coated implants were excluded from the study. Serum and urine samples were collected from fasted participants on two mornings with a 10 ± 3-day interval. Special handling procedures and storage in citrate buffer tubes were required due to the instability of diaminotoluene in biological matrices. Samples extracted with toluene at pH 7–8 and derivatized with pentafluoropropionic anhydride were analysed by GC/MS with negative chemical ionization. The limits of quantification in urine and serum were 10 pg/mL for diaminotoluene and 100 pg/mL for *N*-acetyldiaminotoluene. The limits of detection for diaminotoluene in serum and urine were 2 and 3 pg/mL, respectively, and for *N*-acetyldiaminotoluene 20 and 30 pg/mL, respectively. Patients completed a survey as part of the study. Their demographic data combined with the results of this survey indicated that the subjects were a representative sample of the implant population. There was no detectable 2,4-diaminotoluene, 2,6-diaminotoluene or *N*-acetyldiaminotoluene in sera of either patients or controls at either visit. Unpublished data quoted in the report showed that diaminotoluene is rapidly acetylated in human blood *in vitro*, with a half-life of 15–20 min. The clearance of diaminotoluene in animal studies is rapid, so that the lack of detectable diaminotoluene in serum is not surprising. Quantifiable or detectable levels of diaminotoluene were found in the urine of 48 patients; no quantifiable levels of diaminotoluene were seen in controls, although seven had trace levels of diaminotoluene at one of the two sampling times. There were decreased urinary diaminotoluene levels with increasing time since implantation. After five years, few urine samples had quantifiable levels of diaminotoluene. The urinary data, after normalization for both urinary creatinine and number of implants, were fitted to a concentration versus time curve, the best fit being a one-compartment exponential model. An estimated 'half-life' of 24 months was obtained for biodegradation of polyurethane foam, which is consistent with a recent report of extensive degradation within three years (Sinclair *et al.*, 1993). The estimated half-life suggests that two-thirds of the foam would be degraded within three years

(Hester *et al.,* 1997). [The Working Group noted that the fitting of the data to regression curves involved a variety of common pharmacokinetic assumptions regarding average values assigned to duplicates around the limit of detection, implying that the accuracy of the 'half-life' should be treated with caution.]

In an inhalation study, two male volunteers were exposed to toluene diisocyanate (TDI; isomeric composition 30% 2,4-TDI, 70% 2,6-TDI), in concentrations of 25, 50 and 75 μg/m^3 in air for four hours. Blood and urine were obtained from the subjects at regular intervals after exposure. The samples of plasma and urine were hydrolysed and 2,4- and 2,6-diaminotoluene were determined by capillary gas chromatography. A biphasic elimination of the diaminotoluenes from plasma was observed, with half-time values of 2–5 h (rapid phase) and > 6 days (slow phase). The slowly eliminated fraction may represent protein conjugates of the diaminotoluenes. About 80% of the compounds were excreted in urine within 6 h after the end of the exposure period (Brorson *et al*., 1991).

Potential exposures to 2,4-diaminotoluene arising from polyurethane-coated breast implants have been calculated using a series of assumptions and a range of possible exposures. The polyurethane used in the manufacture of breast implants is a poly(ester urethane) manufactured from polyethylene glycol adipate (PEGA) and toluene diisocyanate. The amount of foam in two breast implants is approximately 2–3 g. PEGA is not a molecule with a discrete chain length but rather a mixture of various chain lengths, and when used in the foam has an average molecular weight of 1500 Da (Amin *et al.,* 1993). The ratio of 2,4-diaminotoluene to 2,6-diaminotoluene in the foam is 4:1 and it is assumed that these isomers are equally susceptible to cleavage, so that 80% of the total diaminotoluene would be 2,4-diaminotoluene (see, e.g., Chan *et al*., 1991b).

The potential annual exposures to diaminotoluenes as a result of implant degradation were calculated based on the 0.8% annual degradation *in vitro* estimated by Benoit (1993), the 30% degradation over nine years reported by Szycher and Siciliano (1991) (assumed to be 3.5% per year) and the total disintegration previously described within seven years (assumed to be 16% per year). It was assumed that the rate of degradation was linear and unaffected by the degree of degradation. The total amount of diaminotoluene in 2–3 g of foam (present in two breast implants) formed from PEGA with an average molecular weight of 1500 Da is 218–328 mg, of which 80% is 2,4-diaminotoluene (175–262 mg). This indicates the range of maximal possible exposure to 2,4-diaminotoluene arising from breast implants.

The potential annual exposure to 2,4-diaminotoluene calculated on the basis of the 0.8% annual degradation (Benoit, 1993) is 1.4–2.1 mg, whereas that based on the 3.5% degradation per year (Szycher & Siciliano, 1991) is 6.1–9.2 mg, and that based on a 16% annual degradation (see above) is 28–41.4 mg.

(*b*) *Wear of dental composites*

Quantification of the chemicals and particles released by chemical dissolution and abrasion of dental composites is complicated. In studies among patients who had

received dental restorations, Dickinson *et al.* (1990) reported a 14- or 26-μm mean annual wear of dental composites (with or without the use of a low-viscosity surface-penetrating sealant), and Freilich *et al.* (1992) reported wear of 45–175 μm after three years, for different composite materials. In a review, different studies were reported to find 7–10 μm mean annual wear (Leinfelder, 1988), while Rasmusson and Lundin (1995) investigated six different composite resin materials and found wear after five years in the range 120–300 μm.

5B.1.2 *Experimental systems*

(*a*) *Polyurethane-coated breast implants*

The release of diaminotoluene from polyurethane foam requires cleavage of two adjacent urethane bonds. Whilst such cleavage has been reported for the poly(ester urethane) foam coating of breast implants, it has not been observed with poly(ether urethanes).

(i) *Toxicokinetics*

A physiologically based pharmacokinetic model for the assessment of carcinogenic risk following degradation of poly(ester urethane) foam from breast implants comprised five compartments: two exiting compartments (kidney and gastrointestinal tract), slowly perfused tissues, richly perfused tissues and a metabolizing tissue (liver). Rats were treated with 2,4-diaminotoluene by intravenous (0.52 mg/kg bw) or subcutaneous injection (0.44 mg/kg bw), or they received a subcutaneous implant of ^{14}C-labelled foam (dose comparable to 80 mg/kg) synthesized from [^{14}C]toluene diisocyanate. The experimental data obtained were fitted to the model. The model assumed a zero-order foam degradation rate constant of 88 ng 2,4-diaminotoluene/g foam per day. 2,4-Diaminotoluene was assumed to be the only degradation product, with a single active metabolite. Almost all of the 2,4-diaminotoluene produced from breakdown of polyurethane foam was assumed to be bound to plasma protein, as was indicated by the experiment with the intravenously exposed rats. The model parameters were optimized with the rat data, and the model was subsequently used to estimate degradation kinetics from an implant by substituting human parameters. A risk estimate was derived based on the plasma levels (Luu *et al*., 1998). [The Working Group noted that the model is limited by the same lack of data on local 2,4-diaminotoluene concentrations at the site of foam degradation that bedevils much of the other published data.]

The toxicokinetics of 2,4-diaminotoluene have been studied in rats and mice after intraperitoneal administration and in rats, rabbits and guinea-pigs after oral administration. The majority of the dose (90%) was excreted in urine after both oral and intraperitoneal administration, except for one study with intraperitoneal administration where 22% of the dose was excreted in faeces between 6 and 16 h after administration. Peak tissue levels were observed at one hour after intraperitoneal administration. The metabolites included mono- and diacetylated amines, a number of unidentified hydroxylated metabolites and products of methyl group oxidation. Little unchanged

2,4-diaminotoluene was excreted after oral administration (Waring & Pheasant, 1976; Grantham *et al*., 1979).

Further toxicokinetic data indicated that acetyl and other acid-labile conjugates are the main metabolites of diaminotoluene in Fischer 344 rats. The monoacetyl derivative was shown to be almost exclusively 4-acetyldiaminotoluene following administration of either diaminotoluene or toluene diisocyanate. Other metabolites included the diacetyl derivative, ring hydroxylation products and both the methyl-oxidized metabolite and its acetylated product. Elimination appeared to be biphasic, with half-time values of 2–5 h for the rapid phase and greater than six days for the slow phase (Bartels *et al.*, 1993).

The distribution of inhaled [^{14}C]toluene diisocyanate in rats was studied over a range of concentrations. Radioactivity was detectable in all the organs studied, but predominantly in trachea, stomach, oesophagus and lung. Virtually all of the radioactivity in plasma was bound to the > 10 kDa fraction, while 41% of the radioactivity in the stomach contents was recovered in this fraction. The results suggest that conjugation is the predominant metabolic pathway of toluene diisocyanate in the rat (Kennedy *et al*., 1994).

Route-dependent differences were investigated in the metabolism of 2,4-[^{14}C]-toluene diisocyanate following oral administration (60 mg/kg) and inhalation exposure (2 ppm, 4 h) of Fischer 344 rats (oral exposure was about 15-fold higher). After oral administration, the majority of the radioactivity was excreted within 48 h, with 81% in faeces and 8% in urine; the tissues and the carcass contained 4% of the dose. In contrast, after inhalation, 47% was found in faeces and 15% in urine, while tissues and the carcass contained 34% of the administered dose. A significant portion of the carcass radioactivity was associated with gastrointestinal contents. Mono- and diacetylated diaminotoluene were detected in urine after oral administration and inhalation exposure, but free 2,4-diaminotoluene was seen only after oral administration. The results suggest different metabolic routes for oral administration and inhalational exposure to 2,4-toluene diisocyanate: after oral administration, 2,4-diaminotoluene is formed, which is readily absorbed; after inhalation, the diisocyanate is conjugated and only small amounts of acetylated 2,4-diaminotoluene are formed (Timchalk *et al*., 1994).

(*b*) *Other polyurethane implants*

Pacemaker leads are prone to two principal degradation mechanisms, metal-catalysed oxidative degradation and environmental stress cracking. There is little release of degradation products, but this degradation modifies the polymer chain, affecting its mechanical properties and leading to failure of its insulation properties. McCarthy *et al*. (1997) reviewed polyurethanes commonly used in this application. They studied degradation of sheets of Pellethane™, Tecoflex™ and Biomer® 18 months after subcutaneous implantation into sheep. By scanning electron microscopy, the surface of Biomer showed uniform pitting and superficial fissuring (< 2.0 μm

depth) whilst Pellethane and Tecoflex surfaces showed severe local embrittlement with fissures up to 40 μm deep. The chemical changes were localized oxidation of the soft segment and hydrolysis of urethane bonds joining the rigid and flexible segments in the polymer structure. There was also localized hydrolysis of urethane bonds within the aliphatic rigid segment of Tecoflex.

The performance and biostability of a poly(ester urethane) arterial prosthesis was investigated *in vitro* and *in vivo*. The material was exposed *in vitro* to either buffer or buffered collagenase and pancreatin solutions for up to 100 days at 37°C. There was an apparent decrease in molecular weight following exposure to the enzyme solutions and a decrease in the concentration of carbonate groups at the surface. This arose from enzyme-catalysed hydrolysis of surface carbonate groups in the soft segment of the polymer, but the enzymes were unable to reach the more hydrolytically susceptible urethane groups in the hard segment (Zhang *et al*., 1994a).

The in-vivo study involved implantation of a thoraco-abdominal bypass in dogs for one or 12 months. At one month, the implant appeared similar to unimplanted prostheses, with a few broken microfibres, but at 12 months the inner surface showed more pronounced degradation, with broken, cracked and fissured microfibres, while the external surface remained similar to that of unimplanted prostheses. This was accompanied by an increase in surface carbonate group and a decrease in urethane group concentrations, especially on the luminal side. This indicates a rearrangement in the microstructure of the polymer (Zhang *et al*., 1994b).

(*c*) *Polydimethylsiloxanes (silicones)*

Silicone polymers contain different sizes of silicone ranging from small amounts of the monomers (low-molecular-weight silicones) to a variety of sizes of polymers (with molecular weights from 7000 upwards; average 30 000).

Silicone polymer is a large cross-linked hydrophobic molecule that is insoluble in water. In various studies the toxicity of the low-molecular-weight precursors of polymeric silicones has been investigated. The patterns of absorption, distribution, metabolism and excretion of these short-chain linear and cyclic siloxanes are related to their size, solubility and lipophilicity.

A sensitive method was developed to analyse low-molecular-weight silicones by use of gas chromatography coupled with atomic emission detection (GC/AED) or with mass spectrometry (GC/MS). Mouse liver homogenate was incubated with silicone oil for 24 h at 25°C, and the extraction efficiency was determined with the above-mentioned methods for each of the components of the oil. Recoveries of 96–98 % were obtained (Kala *et al*., 1997).

The diffusion of low-molecular-weight silicones and platinum – a catalyst used in the preparation of silicone gels – from intact implants into surrounding medium was determined by GC/AED and GC/MS (Kala *et al*., 1997). In lipid-rich medium, leakage of silicones was most prominent, reaching rates of 10 mg/day per 250-g implant at 37°C. Platinum levels in silicone implant gels were determined to be

175 μg per 250-g implant. Platinum diffused from the intact implant into lipid-rich medium at a rate of 20–25 μg/day/250-g implant (Lykissa *et al*., 1997).

The toxicity of decamethylcyclopentasiloxane (D_5), used as an intermediate in the production of silicone polymers, was tested in a three-month nose-only inhalation experiment with Fischer 344 rats. The dose range was 0–224 ppm, given for 6 h per day on five days per week. At the high dose, an increase was noted in the serum levels of γ-glutamyltranspeptidase in both sexes, and of lactate dehydrogenase in females. Absolute and relative weights of the liver were increased in both sexes at the end of the exposure period, but this effect was resolved after a one-month recovery period. Focal macrophage accumulation and interstitial inflammation were noted in lungs of male and female rats after exposure to the highest dose. These effects appeared not to be entirely reversible after the recovery period (Burns-Naas *et al*., 1998).

With increasing molecular weight, the solubility and oral absorption of the siloxanes decrease, reaching essentially zero for molecules containing eight or more siloxy units. Half-lives of short-chain siloxanes were reported to be of the order of hours or days. Metabolic demethylation of short-chain silicone precursors does occur, but in no case has the loss of more than two methyl groups been shown, and there is no evidence for demethylation of silicone polymer. Metabolism of siloxanes to silicates has not been demonstrated (Lykissa *et al*., 1997; Kala *et al.*, 1997).

A series of studies have used nuclear (^{1}H and ^{29}Si) magnetic resonance (NMR) and ^{1}H localized spectroscopy to assess the migration and biodegradation of silicone in rats that had received silicone gel-filled implants. There was no evidence for the presence of silicone in the liver until up to six months after implantation. On the basis of ^{29}Si-NMR data obtained nine and twelve months after implantation, it was concluded that migration of silicone to the liver and formation of new silicon-containing compounds (probably silica gel and high coordinated silicon complexes) had occurred. Only limited quantification of silicone metabolism was given and no identification was provided of the chemical structure of the products formed. The authors did not report the use of reference standards for spectroscopic analysis (Garrido *et al*., 1993; Pfleiderer *et al*., 1993a,b; Garrido & Ackerman, 1996). The findings could not be confirmed by others, and a number of technical shortcomings in the methodology have been pointed out (Dorne *et al*., 1995; Macdonald *et al*., 1995; Taylor & Kennan, 1996).

[The Working Group noted that the available data did not provide convincing evidence that degradation of silicones occurred.]

(*d*) *Degradable polymers*

Flat plates of ε-caprolactone–L-lactide copolymer [50:50 (w/w), molecular weight 162 kDa; 20 × 10 × 1 mm in size] were implanted subcutaneously into 50 male Wistar rats. The copolymer was synthesized by bulk ring-opening polymerization of the two components at 190°C for 5 h followed by precipitation from a dichloromethane solution in methanol and drying under reduced pressure. The positive control implant was a 1-mm thick plate of medical-grade polyethylene prepared to identical dimensions on the

same machine with no additional chemicals used during processing. Encapsulation of the copolymer implants had occurred by one month, but degradation had also started. The molecular weight decreased to 36% of the initial value within one month, and to 31% by six months. Macroscopically, the implants retained their initial shape up to six months, at which time the surfaces were roughened but no tissue in-growth was observed. After six months, it was not possible to separate the implant from surrounding tissue. By 12 months, the plates had broken into several pieces and by 18 months they had degraded into small fragments with diameter between 10 and 200 μm. The tissue reaction around the fragments became more marked as degradation proceeded, but remained confined within the tissue capsule. By 24 months, fragments were being resorbed and capsules averaged $10 \times 5 \times 0.5$ mm in size with the broken fragments located in a $3 \times 2 \times 1$ mm volume in the centre of the capsule (Nakamura *et al*., 1998).

The same group (Nakamura *et al*., 1994) had earlier compared tumorigenicity of plates of poly-L-lactide and medical-grade polyethylene with identical dimensions in a similarly designed study. The viscosity-average molecular weight decreased from 25.3×10^4 initially to 4.97×10^4 at six months, 4.50×10^4 (18% of original) at 12 months and to 2.12×10^4 (8% of original) at 24 months. However, the plates still retained their initial size of $20 \times 10 \times 1$ mm at 24 months although white spots had begun to appear within six months and scanning electron microscopy at 24 months revealed holes with diameters of several micrometres. Clear areas of poly-L-lactide were homogeneous, whereas the white spots had developed a porous structure.

A similar study of plates of polyglycolic acid showed complete absorption within two months (Nakamura *et al.*, 1997).

The synthetic α-polyesters are synthesized from α-hydroxy acids such as glycolic and L-lactic acid. As the D-isomer of lactic acid is not easily metabolized by humans, only the L-isomer is used in biomedical applications. Suitable polymers are obtained by ring-opening polymerization of the cyclic diesters, i.e. the lactide and glycolide. The mechanical properties and the degradation characteristics of biodegradable implants of these materials depend on a number of factors, such as polymer synthesis and purification, the structure of the polymer chains, and the shape, surface roughness and pore size of the implant. Various methods to control the porosity of the polymer have been developed. The degradation rate of synthetic α-polyester implants may vary from a few weeks to over a year and can be modulated by appropriate polymer selection and control of manufacturing conditions. The degradation rate during the early stages of bone healing is of particular importance, because the implant material should be stable enough to act as a substrate for bone-forming cells. Furthermore, an active growth-inducing effect of the implant on bone cells can be achieved by inclusion of growth factors in the implant formulation (Coombes & Meikle, 1994).

(*e*) *Substances released from dental composites*

After storage of a well-cured composite specimen for one year in water, analysis by high-performance liquid chromatography (HPLC) showed that several substances

had been released. However, none of the chromatographic peaks represented an organic substance known to be present in the polymer, except for possible traces of triethyleneglycol dimethacrylate (Ruyter, 1995).

Methacrylic acid is released from all resin systems. Di- and monomethacrylates hydrolyse to methacrylic acid and the alcohol component at neutral pH catalysed by an unspecific esterase (hydrolase) and by enzymes in the saliva. The rate constants of enzymatic hydrolysis of various (di)methacrylates increase in the following order: 2-hydroxypropyl methacrylate (HPMA) < 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy)phenyl]propane (bisphenol A diglycidylether methacrylate; bis-GMA) < lauryl methacrylate (LAMA) < decyl methacrylate (DECMA) < triethyleneglycol dimethacrylate (TEGDMA) < 1,6-bis-(methacryloyloxy-2-ethoxycarbonylamino)-2,4,4-trimethylhexane (UEDMA) < diethyleneglycol dimethacrylate (DEGDMA). Esterase added to aqueous slurries of various powders made of polymerized bis-GMA/TEGDMA mixtures liberated methacrylic acid, presumably resulting from degradation of dimethacrylates bonded only in the matrix by one end of the molecule. It has been proposed that hydrolases in saliva increase the wear rate of composite resin fillings. The hydrolytic activity of saliva is believed to depend on the activity of esterases or hydrolases released from various types of microorganisms, and repeated measurements from various collections of saliva from the same person showed some variation (Munksgaard & Freund, 1990).

The results described above are consistent with previous assumptions that hydrolases from bacteria in the mouth contribute to a breakdown of the substances contained in fixed and removable dentures, such as methyl methacrylate (Engelhardt & Grün, 1972).

Formaldehyde release from dental composite resins immersed in water for 72 h at 37°C ranged from 0.05 to 0.5 μg/cm^2 for different materials. The highest releases were observed with chemically activated materials with inhibition layers, and in one visible-light-activated material. Both grinding and polyester coating of the specimens reduced the release of formaldehyde. Only small or insignificant differences in release of formaldehyde were observed between ground specimens and specimens coated with a polyester film. The release of formaldehyde decreased with time, but it was still detectable after 115 days of immersion in water. The highest quantities of formaldehyde were released by specimens with thicker inhibition layers. As the half-life of formaldehyde is approximately 1.5 minutes, formaldehyde released from dental materials prepared according to technical standards will not reach local concentrations of toxicological relevance. However, the observed concentrations of formaldehyde may be sufficient to elicit allergic reactions (Øysæd *et al*., 1988).

Bisphenol A and bisphenol A dimethacrylate are monomers of certain dental sealants. A sealant based on bis-GMA showed oestrogenic properties in human MCF7 breast cancer cells by inducing cell proliferation and progesterone receptor expression. In contrast, three resin-based dental composites did not induce proliferation of MCF7 cells. Saliva samples were obtained from 18 patients during 1-h periods before and

after they had received applications of 50 mg of the bis-GMA sealant on their molars. Bisphenol A (90–931 μg) was identified in all post-treatment saliva samples, and its methacrylate derivative in 3 out of 18 samples. Samples containing the highest amounts of bisphenol A and bisphenol A dimethacrylate stimulated MCF7 cell growth (Olea *et al*., 1996).

5B.2 Tissue responses and other expressions of toxicity

5B.2.1 *Humans*

(*a*) *Polydimethylsiloxanes (silicones)*

Much attention has been paid to assessment of the long-term effects of implanted silicones, mainly following their use for breast augmentation. Only limited data are available on the effects of silicones used for other applications, although several complications following implantation of silicone elastomer prostheses have been reported. In a case series of 94 patients with 'Swanson' silicone elastomer implants, mainly finger joints, followed up for a mean of 116 months, an intact osseous bed was detected radiographically in only 41% of patients, with osteolysis or distant bone cysts evident in the remainder. The extent of these effects was correlated with the duration of implant. Histology of 11 revisions revealed silicone particles and an aggressive histiocytic response, with foreign-body giant cells (Wanivenhaus *et al*., 1991).

In a case series of 422 augmentation rhinoplasty patients with silicone nasal implants over 10 years, the few late complications were predominantly aesthetic (Deva *et al*., 1998).

A case was reported of deterioration of a silicone elastomer toe implant leading to an intense foreign-body granulomatous reaction. The implant was fragmented five years after implantation and a surrounding mass with multicystic changes was present. This was diagnosed as a florid granulomatous reaction that presented as a tumour-like condition, possibly subsequent to avascular necrosis of the first metatarsal head (Ognibene & Theodoulou, 1991).

Another case, classified as 'silastic synovitis' in the great toe was reported following implantation with a polyamide (Dacron)-coated silicone elastomer prosthesis eight years earlier (Glod & Frykberg, 1990) and a further case of cystic osteolysis and detritic synovitis was reported two years after placement of a silicone elastomer implant in the wrist. A granulomatous inflammatory response, with foreign-body particles, was noted, involving histiocytes and giant cells with numerous filopodia that resembled osteoclasts by electron microscopy. A histiocytic reaction to silicone particles was postulated as the cause of osteolysis (Ekfors *et al*., 1984). These studies suggest that normal foreign-body reactions operate in response to silicone elastomer and reveal no specific preneoplastic changes.

Further data have come from studies on silicone breast implants and the tissue response to these implants. A series was reported of 15 resected breast implant capsules, removed from 11 post-mastectomy and four breast augmentation patients. Of these, seven capsules showed a capsule–implant interface lined by a single layer of

epithelial cells. Implantation time for these was 36–240 months (median, 60; mean, 100); three were textured and four were smooth-surface implants. The layer of epithelioid cells showed characteristics of true synovial cells, with an occasional multinucleated cell and normal-appearing mitotic figures. Immunoreactivity of synovial cells for macrophage lineage suggested they were of histiocytic origin. The metaplastic synovial cell response was similar to that reported in bone or soft tissues in contact with other materials. The presence of silicone in the capsules was demonstrated. Of the eight other capsules, six were entirely acellular fibro-collagenous membranes of variable thickness. The other two were acellular membranes with diffuse microcalcification. Implantation times were not given. The authors suggested that synovial metaplasia was an adaptive response to silicone gel leakage and prosthesis movement (del Rosario *et al*., 1995). Although seen in this limited study in association with both smooth- and textured-surface implants, synovial metaplasia has been reported particularly in association with textured implants (Bleiweiss & Copeland, 1995).

Explanted capsular tissue was also studied in a series of 86 cases: 50 silicone gel-filled (46 smooth-surface, four textured), 12 double lumen (one textured), 14 saline-filled (all textured; four tissue expanders), nine poly(ester urethane)-coated and one injected silicone. Capsule pathology was described in some detail and was typical of a chronic inflammatory response. Calcification was seen in 11 capsules, all associated with the presence of implant stabilization patch material and with smooth-surface implants, 10 of them gel-filled. Vacuoles presumed to contain silicone were seen in macrophages in 68 cases. The presence of silicone was confirmed by infrared spectroscopy in 55/76 cases and by Raman microspectroscopy in 15 cases. Vacuolated macrophages formed sheet-like aggregates and were associated with multinucleated giant cells. Synovial metaplasia, with formation of a pseudoepithelium, occasionally with micropapillary structures, was noted in 38 cases, irrespective of filling type but more frequently with textured surfaces. Seven of these were examined immunohistochemically and showed a histiocytic phenotype, staining positive for CD68(KP-1) or lysozyme in six of seven cases (Luke *et al*., 1997).

A further description of capsule pathology was derived from a series of 71 explantations of silicone gel breast implants and a review of data on the pathological response to silicone leakage. Granuloma formation was associated mainly with extravasation of gel or with silicone injection, giant cells and foam cells being associated with the former. Phagocytosis of silicones in the lymph nodes by multinucleated giant cells was also described. Synovial metaplasia was characterized as changes in the innermost layer of fibroblastic cells surrounding the implant, resembling normal joint synovium. A layer, one to seven cells thick, of large, mostly polygonal epithelioid cells of various sizes was seen. Both phagocytic and secretory cells were noted, together with a prominent reticulin network; marked staining was observed for eosin and Alcian blue and, immunohistochemically, for vimentin (Beekman *et al*., 1997; van Diest *et al.*, 1998).

In the case series of 86 patients described above (Luke *et al*., 1997), the presence of talc in tissue surrounding breast implants, presumably arising from the use of surgeons' gloves, could be a significant confounding factor with many implant types. Talc was identified in 42 cases (intracellular) and 14 cases (extracellular), a total of 65% of cases, irrespective of filling type. The identity of talc was confirmed by scanning electron microscopy/energy dispersive X-ray analysis of peaks for O, Mg and Si and by comparison of infrared spectra. Cells containing talc were identified immunohistochemically as macrophages. The significance of this finding in promoting peri-implant fibrosis, a common complication in silicone breast implants, remains to be determined.

[The Working Group noted that the inflammatory and metaplastic changes observed in these studies with breast implants are consistent with a foreign-body reaction and revealed no specific preneoplastic changes.]

(*b*) *Polyurethane-coated breast implants*

In a series of 86 cases (described above) (Luke *et al*., 1997), the cellular response was found to be more prominent with the nine polyurethane-coated implants than with uncoated silicone shells. The reaction featured vacuolated macrophages, a chronic granulomatous inflammation and multinucleated giant cells, some with asteroid bodies. Two different chemical species of polyurethane were identified by infrared spectroscopy.

Two cases of haematoma were reported, six months and three years after implantation of polyurethane-coated breast implants. The authors suggested that the etiology was related to a highly vascular inflammatory response to the polyurethane coating (Wang *et al*., 1998).

(*c*) *Polytetrafluoroethylene implants*

Granulomatous responses were noted in a case series of eight cancer patients in whom polytetrafluoroethylene paste (Teflon 50% w/w in glycerine, Mentor) had been injected into the larynx to restore the voice. A further three cases involving the use of the same product for this purpose have also been reported. Chronic inflammatory foreign-body responses were seen, with fibrosis and encapsulation or granuloma formation, but there was no evidence of metaplasia or neoplasia (Stone & Arnold, 1967; Harris & Hawk, 1969; Wenig *et al*., 1990).

(*d*) *Joint replacements, polyethylene and bone cement*

With cemented joints, which make up the majority of joint implants, bone and surrounding tissues are exposed to acrylic substances that cure *in situ* in an exothermic reaction (a temperature rise of up to 17°C has been reported by Reckling & Dillon (1977)). This inevitably gives rise to release of monomers or additives into surrounding tissue. Wear of articulating surfaces leads to release of particles of varying size, shape, surface characteristics and composition. These circumstances, and the physical stresses

related to the load placed on the implant during use, imply that there is a particularly demanding biological environment. Due to concern over the long-term performance of joint replacement prostheses, numerous studies have examined the tissue response to these implants, with special emphasis on determining mechanisms of necrosis, bone resorption or other factors leading to joint failure. Few of these studies have specific relevance to carcinogenicity but they do illustrate the pathological processes that occur in response to the various materials that are used in orthopaedic joint replacement surgery.

The sort of response commonly seen is typified by a series of 23 bone specimens obtained after one month to seven years of exposure to acrylic cement (four specimens were retrieved at five to seven years after implantation). The topographic anatomy of the sites of new and old bone, of fibrocartilage and of fibrous tissue at the cement–bone interface was described. From the distribution of these tissues, an attempt was made to interpret how the load of body weight is transmitted from the cement to the shaft of the femur. Fibrocartilagenous layers produced in response to mechanical pressure were noted, with occasional points of direct contact between cement and bone. Ossification was noted in the vicinity of underlying bone and a foreign-body giant-cell response was seen on the surface of fibrous tissue in direct contact with cement. The latter response appeared to be most prominent at two to five years (Charnley, 1970).

The histological and immunohistochemical characteristics of tissue surrounding the acetabular component were reviewed in a case series of 11 hip revision patients. The fibrous and inflammatory responses were assessed quantitatively. Cathepsin-G activity, associated with monocyte or macrophage-like and fibroblast-like cells, was higher in periprosthetic tissue than in control tissue from synovial capsule or pseudosynovial fluid. The authors suggested that cathepsin-G, which is known to interact with TNF-α and other enzymes, has a role in the loosening of the prosthesis (Takagi *et al*., 1995).

Information on allergies or hypersensitivity reactions to non-metallic constituents of orthopaedic devices is scarce. The sensitivity of 25 patients undergoing orthopaedic surgery was studied by collecting venous blood at short intervals during the surgery and measuring serum concentrations of total haemolytic complement and of components 3 and 4. Eleven patients received total hip arthroplasty (no cement used), while two groups of seven patients were treated for hemi-arthroplasty with and without the use of methyl methacrylate cement, respectively. Neither the surgery nor the use of the cement induced activation of the complement (Monteny *et al*., 1978).

(*e*) *Dental materials*

In general, dental materials have acceptable biocompatibility for clinical use in patients. Methyl methacrylate monomer and mercury have in a few instances of high occupational exposure led to classical, dose-dependent toxicological problems. With these exceptions, the amounts of individual chemicals to which professionals and patients are exposed do not seem sufficient to cause manifest, systemic toxic effects.

Localized effects of dental materials such as those related topographically to oral lichenoid lesions may have a premalignant potential. These lesions require adequate diagnosis and systematic clinical follow-up (Holmstrup, 1999).

(i) *Irritant contact dermatitis*

This acute toxic reaction is a dermal inflammatory response to primary irritants. It is a result of physical or chemical action due, for instance, to trauma, ionizing radiation, heat, bases, acids or other reactive chemicals. Depending on the concentration and exposure time, the reaction can vary from erythema to necrosis. The substances may exert a direct cytotoxic effect on the cells in the superficial skin or mucosa, most often corresponding exactly with the site of application. In the oral cavity the boundaries of the inflamed area may be more diffuse. This type of reaction is seen when, e.g., phosphoric acid enamel etchant or bonding agents are inadvertently spilled on the mucosa or skin and remain there for some time (Jacobsen *et al.*, 1991).

Cumulative insult dermatitis can develop following repeated contact with low doses of primary irritants over extended time periods and is caused by a gradual deterioration of the natural barriers. Such exposure conditions are mainly seen in occupational settings. The changes in skin or mucosa are localized to the area of contact with the offending agent and they do not spread to other sites. The diagnosis of cumulative insult dermatitis or mucositis cannot be made on the basis of epicutaneous patch testing or other investigations, but is made by exclusion of other possibilities, based on the case history, the clinical appearance of the lesion and negative patch tests. One example is the 'three-finger syndrome' with a clear positive topographical relationship between the skin changes and contact with dental materials in persons who have negative patch tests to the constituents of the relevant dental materials. This type of reaction is often seen on the first three fingers of the left hand, in right-handed persons. These three fingers are exposed to spray from bonding resins when used to reflect the patients' lips during treatment and may also have been in contact with the remnants of spills on the outside of squeeze-bottles containing the liquid monomers (see, e.g., Munksgaard, 1992).

Gloves used for prevention of microbial contamination do not protect from exposure to monomers in dental materials. The monomers penetrate vinyl and latex gloves within a few minutes, and may therefore be in contact with the skin for an extended time period (Munksgaard, 1992).

Paraesthesia related to contact with dental resins has been observed in a few instances. Dental technicians and orthopaedic surgeons may have dermatitis associated with the use of methyl methacrylate monomer, often in the form of marked dryness and fissuring of the skin. A unique feature is a paraesthesia of the fingertips in the form of a burning sensation, tingling and slight numbness. This type of paraesthesia was observed in two orthodontists who had become sensitized to the monomer in orthodontic bonding materials (Fisher, 1982). This might be due to a direct neurotoxic effect of the methyl methacrylate monomer (Seppäläinen & Rajaniemi, 1984).

Biopsies from a dental laboratory technician who had been preparing dental prostheses for more than 30 years have shown direct pathological effects of methyl methacrylate on nerve fibres, resulting in a sensorimotor peripheral neuropathy (Donaghy *et al*., 1991).

(ii) *Allergic contact dermatitis*

Although allergic reactions are basically different from toxic reactions, their clinical manifestations are often similar or even identical. Most components of dental materials are of low molecular weight. By acting as haptens and combining with body proteins, they may form complete antigens capable of inducing sensitization of immunocompetent cells. The risk of sensitization varies, depending on the type and concentration of the substance and the type and condition of the contacting tissues. The actual contact site with the allergen is usually the first place where clinical symptoms develop. However, contact-sensitized individuals may develop a number of symptoms when exposed to the allergen systemically, either orally or by inhalation, infusion or transcutaneous or transmucosal absorption.

The problems related to systemic allergic contact dermatitis are complex and not completely understood (see, e.g., Nakada *et al*. (1997) for a discussion of allergic reactions to gold chloride, mercuric chloride and metallic mercury). Allergic reactions to any component of dental materials may occur in patients and in dental professionals handling the materials. More than 130 common allergens have been identified among the various dental materials available today (Kanerva *et al.,* 1995).

(iii) *Anaphylactoid reactions*

Anaphylactoid reactions in children have been reported following the placement of fissure sealants, which are based on the same ingredients as composite materials (Hällström, 1993). An anaphylactoid reaction developed in a four-year-old child after contact with a dental surgeon's latex gloves (Rasmussen, 1997).

(iv) *Photo-related reactions*

Phototoxic or photoallergic reactions have not been documented in the context of oral medicine, but may well represent a new occupational problem as a result of the extensive use of powerful light units in the curing of dental resin-based materials. Substances of dental interest which may have phototoxic properties are sulfonamides (present in some cavity liners), phenothiazines, griseofulvin and some tetracyclines. Examples of photoallergic compounds of interest in the context of dental treatment are eugenol, chlorhexidine, derivatives of *para*-aminobenzoic acid, eosin (a colorant in some lipsticks), sulfonamides and phenothiazines. A generalized, intensely erythematous eruption of the face and submental area in a dental hygienist was traced to a combination of long-term trimethoprim medication and exposure to stray light from a laboratory photocuring unit (Hudson, 1987). The possibilities of photo-related reactions should be taken into account in evaluating dermatoses in dental personnel and patients.

(v) *Contact urticaria*

There have been many reports of patients with urticarial reactions to dental materials. Contact urticaria is a wheal and flare response elicited by the application of various compounds to intact skin. One case of persistent generalized urticaria was traced to a resin-based orthodontic bonding agent (Tinkelman & Tinkelman, 1979). Another case also presented traits of an anaphylactoid reaction (Hallström, 1993).

Immunological contact urticaria is an immunoglobin E-mediated reaction, entailing histamine release from mast cells. It may be localized or widespread and is sometimes associated with features of anaphylaxis. Such reactions have been observed after contact with surgical latex gloves, where both the powder and the latex may contain substances capable of eliciting urticarial reactions (Wrangsjö *et al.*, 1988).

Non-immunological contact urticaria is clinically indistinguishable from the other variety, and occurs without previous sensitization in most exposed persons. The reaction remains localized and does not spread to become generalized urticaria, nor does it cause systemic symptoms. Its pathogenetic mechanism is not clearly understood. This type of contact urticaria may be elicited by a number of compounds, notably benzoic acid, which occurs naturally in many fruits, is added as a preservative (E 210) in salad dressings and other processed foods and is formed as a degradation product of benzoyl peroxide, used as an initiator in dental composites and denture base resins (Koda *et al.*, 1990).

(vi) *Other types of hypersensitivity: hyperreactivity and intolerance reactions*

These types of 'other hypersensitivity reaction' have been studied considerably less than those mentioned above. A fairly large proportion of hypersensitivity problems must still be ascribed to the 'nonallergic type with unknown cause', since there is limited information about which cells and mediators are involved (SOU, 1989).

Hyperreactivity is associated with vasomotoric reactions of the airways and eyes. Vasomotoric rhinitis/conjunctivitis simulates a chronic allergic condition, but may be due to a direct influence on the peripheral nerve endings in the mucosal linings. Reactions may be elicited by certain perfumes, including eugenol, volatile monomers, fumes from soldering fluxes containing colophony and other irritants.

Intolerance reactions may simulate allergic reactions, but are not mediated by the immune system. These reactions are associated with insufficient levels of enzymes that normally metabolize substances such as fructose, sucrose, acetylsalicylic acid, ethanol and benzoic acid. Whether the small amounts of benzoic acid liberated from dental resins (Koda *et al.*, 1990) may contribute to such problems is not known.

(vii) *Local and systemic effects*

Acid etching and adhesive agents

The reactions of the dental pulp to acid etching in combination with different bonding procedures before implantation of composite restorations have been studied

in human teeth *in vivo*. The experimental restorations were placed in intact premolars of 11–15-year-old children. The teeth were scheduled for extraction for orthodontic reasons, which took place four months after the treatments. Acid etching of the dentin appeared to increase the penetration of a low-viscosity resin into the dentinal tubules, thus enhancing the adverse effects of this resin, unless the dentin had been protected during the etching procedure. A glycidyl methacrylate-based dental adhesive did not cause pulpal reactions. The effects in the dental pulp, e.g., inflammatory reactions and growth-reduction of odontoblasts, could only partly be explained by a direct toxic effect of the material tested. Other factors, such as tooth position and marginal leakage resulting in bacterial in-growth also contributed to the inflammatory reaction (Qvist *et al.*, 1989).

In a similar study in premolars in children, histological observation of dental pulp revealed that a glutaraldehyde-based dentinal adhesive caused slight to severe responses, changes in odontoblastic layers and inhibition of dentinogenesis after periods of up to 120 days (Elbaum *et al.*, 1991).

The dental pulp of eight permanent premolars and molars was protected during dental restoration by direct capping with a glutaraldehyde-containing dentin adhesive. All eight teeth examined remained vital and without symptoms during an initial observation period of up to six months after treatment (Heitmann & Unterbrink, 1995).

A review on the biocompatibility of dental bonding agents reported that some agents seem to be irritant to pulpal tissue, and recommended pulpal protection, preferably in the form of spot lining in the deepest part of the cavity (Al-Dawood & Wennberg, 1993).

Composite filling materials

The inherent polymerization shrinkage of composite resins may allow bacterial leakage and give rise to pulpal reactions. Pulp tissue was investigated from exfoliated primary teeth in which shallow class II composite restorations without bonding resin had been placed eight months to more than six years earlier. Histological examination revealed no pulpal inflammation in 5/16 teeth, moderate to severe inflammation in 4/16 teeth, and pulpal necrosis in 7/16 teeth. These pulpal effects paralleled the extent of bacterial penetration into the pulp (Varpio *et al.*, 1990).

In a review of the pulpal response to composite restorations, it was confirmed that the toxicity of the materials used was of less concern than bacterial invasion into the dentinal tubules (Barnett, 1992).

From a summary of clinical reports on dental composites, it appeared that pulp inflammation has not been adequately documented in clinical trials, most studies using sensitivity responses as a measure of biological compatibility. However, sensitivity does not seem to have any correlation with pulpal inflammation, which is caused by mechanical, thermal, chemical and bacterial insults (Bayne, 1992).

A comparable occurrence of post-treatment sensitivity was observed between teeth restored with composite filling materials (25%) and those with dental amalgam (21%).

A quarter of the patients accounted for 60% of complaints, leading the authors to suggest that three factors might contribute to the increased sensitivity: the material, the operating procedure and the pain threshold of the patient. In no case was sensitivity reported after six months (Borgmeijer *et al.*, 1991). In another study with a five-year observation period, only one out of 176 restorations was replaced due to increased sensitivity (Rasmusson & Lundin, 1995).

On the basis of surveys among Scandinavian dentists, it can be estimated that adverse reactions of patients to composite materials occur at frequencies of 1:1000 after prosthodontic treatment and 1:10 000 after paedodontic treatment (Henstен-Pettersen & Jacobsen, 1991).

In a recording by 137 dentists over a 10-day period of side-effects of dental materials in clinical practice, no acute reactions were reported from 2400 composite restorations (Kallus & Mjör, 1991).

Lichenoid reactions related to composite restorations were observed in a study of 17 patients, eight of whom received dental composites to replace amalgam restorations that were topographically related to earlier lichenoid lesions. In addition, lichenoid reactions were seen in nine patients with no previous history of such reactions. Total remission in four cases and partial remission in five patients were observed after the composite material had been replaced with gold inlays or porcelain fused to gold crowns (Lind, 1988).

In a study on experimental gingivitis, no difference in the development of plaque and gingivitis was observed on intact enamel and composite fillings during a seven-day period (van Dijken *et al.,* 1987).

Dust particles generated from composite materials during grinding and finishing composite restorations were examined. About 60–80% of the particles trapped on filters were respirable (size 0.5–5.0 μm) and were composed of 70–100% silica. Dust generated from composites containing crystalline silica as a filter was suggested to have potential to cause silicosis, whereas dust generated from composites containing amorphous silica was not expected to have the same potential (Collard *et al.*, 1989, 1991).

5B.2.2 *Experimental systems*

(*a*) *Inflammatory, hyperplastic and metaplastic responses*

(i) *Polydimethylsiloxanes (silicones)*

The inflammatory response to silicones is well characterized and is predominantly lymphocytic, with macrophage involvement and cytokine production; the outcome is normally fibrotic with occasional granuloma (sometimes termed siliconoma) formation. Animal studies have shown that silicones are not significantly immunotoxic but some can act as adjuvants under experimental conditions; however, their ability to elicit specific antibody responses or cell-mediated immunity is limited. Silicones can be found in tissues following implantation of gel or liquid, in deposits ranging from large intercellular or cytoplasmic droplets to microscopic particles within macro-

phages. Transport of silicones via the lymphatic system has been demonstrated (Rees *et al*., 1970; Tinkler *et al*., 1993; Gott & Tinkler, 1994; Shanklin & Smalley, 1995; Marcus, 1996).

With the exception of those investigating foreign-body carcinogenesis or using silicones as control materials, few studies have looked into relevant tissue responses arising from the presence of silicone materials. One study examined the reaction to various components of silicone breast implants in rats. Discs (3/8 × 0.02 inch (9.5 × 0.5 mm)) of various silicone gel breast implant component materials (elastomer shell, xylene-extracted shell, silicone extract (coated onto an implant shell), silica-free silicone) were implanted subcutaneously into groups of three Lew/SsN rats (two discs per rat). Fumed silica (approximately 1 mL) and the liquid silicone oil and viscous gel (1 mL) were injected subcutaneously. Histological examination at 7, 14, 28, 56 and 90 days showed the progression of the inflammatory and fibrotic response and the differences between the materials. The degrees of fibrous capsule formation and migration of silicone were dependent on molecular weight. The response also varied with the degree of compliance of the material, which coincided with fumed silica content. Free-fumed silica induced an intense early response, with fibroblasts, pericytes, macrophages and some surrounding mast cells. Exudate was seen between the capsule and the material together with some neovascularization. Later, cell destruction and the absence of a fibrous capsule were evident, with fibroblasts, lymphocytes and macrophages but no multinucleated giant cells. The authors concluded that there was an immunological component to the response with the silicone extract, based on the strong multinucleated giant-cell response; however, the reported results did not indicate such a response with fumed silica. No other cellular response was noted and there were thus no indications of pre-carcinogenic mechanisms; however, the number of animals and the duration of the study were inadequate to allow firm conclusions to be drawn (Picha & Goldstein, 1991).

(ii) *Polyurethanes*

Highly stable poly(ether urethanes) are now used in most implant applications (e.g., in vascular grafts). However, most investigations into the effects of implanted polyurethanes have concentrated either on estimating the risk arising from degradation of the poly(ester urethane) foam coating in certain breast implants or on assessing the ability of this material to initiate foreign-body carcinogenesis (see Section 5B.1). One study has investigated the response to the poly(ester urethane) used as a breast implant coating material. Four poly(ester urethane) foam discs (6 mm diameter × 3 mm; soaked in ethanol and rinsed) were implanted into pockets between the mammary fat pad and muscle of groups of 25 female B6D2F_1 mice. Controls received sham surgery. Mice were killed at 10 time points between one day and 47 weeks for limited histopathological examination and, at four weeks, for electron microscopy. The inflammatory response observed involved macrophages and giant cells, leading to fibrosis. Phagocytosis of polyurethane particles was noted from week 4 and the implants had

virtually disappeared by week 47. No inflammatory or other changes that might indicate a predisposition to carcinogenicity were observed (Devor *et al*., 1993).

A group of 132 female Swiss albino mice were given sub-mammary implants of poly(ether urethane) foam discs (5 mm diameter × 2 mm) and observed for one year. The relatively large pore size used in the foam (40–45 interconnecting pores per inch (16–18 per cm)) was associated with ingrowth of fatty and loose connective tissue, with a small amount of dense collagenous tissue (Dunaif *et al.*, 1963).

(iii) *Polyethylene*

Because of its widespread use in orthopaedic joint implants and the associated clinical damage due to the effects of wear particles, ultra-high-molecular-weight polyethylene has been investigated in many experimental studies on the biological effects of wear debris. These studies give insight into inflammatory processes and their mediators and into the mechanism of necrosis or osteolysis associated with prosthesis loosening. However, the relevance of such studies to carcinogenicity is limited and they are not discussed here.

Male and female Fischer 344 rats were given subcutaneous implants of 65 mg particles (size range, 425–600 μm) of polysulfone (29 rats) or polyethylene (32 rats) dispersed in 0.5 mL saline. Fibrous capsule tissue from around the implants was examined to determine the proportion of particle surface associated with the various inflammatory cell types. Rats were given the implants at 16 weeks and were killed at 100–118 weeks. A group of 26 controls received sham operations. There was no discernible difference between the two polymers in terms of the macroscopic, microscopic or quantitatively measured response. Almost half of the particle surface was covered by macrophages, while giant cells and fibrous tissue each covered about 20%. In a zone around each type of polymer particle, comparable numbers of giant cells were found (see Table 56). Surface texture influenced the adherent cell type: rough particles were predominantly covered by giant cells, whereas smooth particles were more often associated with macrophages or fibroblasts. This finding reached statistical significance, on the basis of a [presumably subjective] assessment of roughness at × 100 magnification. Cell morphology was described from a transmission electron

Table 56. Surface coverage of polysulfone and polyethylene particles by different cell types

Material	Mean particle surface covered by cell type (%)			No. of giant cells
	Macrophage	Giant cell	Fibrous tissue	
Polysulfone	49	21	17	2
Polyethylene	47	18	23	3

Adapted from Behling and Spector (1986)

microscopy study. No differences were found in the ultrastructure of the cells surrounding the polyethylene and polysulfone particles. Numerous cell processes in interfacial macrophages, evidence of phagocytic activity, amorphous extracellular material close to the polymer surface and occasional collagenous material but no fibroblasts at the material surface were observed (Behling & Spector, 1986).

(iv) *Polytetrafluoroethylene*

Groups of 50 Sprague-Dawley rats received 0.1-mL subcutaneous injections of particulate plastics, namely 50% polytetrafluoroethylene in glycerine (90% of the particles were smaller than 40 μm) or a 38% silicone elastomer in hydrogel carrier (most particles were 100–150 μm in size, but some were 5 μm). Controls were untreated. Injection sites and major organs were examined histologically after two years. A normal chronic inflammatory response was seen but no inflammatory infiltrate in the tissue adjacent to the injection site (Dewan *et al*., 1995a).

The presence of polytetrafluoroethylene beads in the urinary bladder resulted in accelerated cell turnover in a rat model in which the bladder was transplanted into muscle to avoid problems associated with implantation into the bladder (heterotopically transplanted bladder system). Fischer 344 rats in which urinary bladders from syngeneic rats had been transplanted into the gluteal muscle were used, four weeks after the transplantation. Two 6-mm diameter beads of polytetrafluoroethylene were placed in the bladder lumen. One group of 25 rats received the beads and one group of 25 rats had a sham operation. Bladders were filled with saline containing gentamicin after the operation. One week later, groups of rats received either 0.5 mL rat urine or 0.5 mL saline weekly in their transplanted bladder. Histological and autoradiographic ([^{3}H]thymidine incorporation) examination took place one, three or six weeks after bead implantation. Moderate foci of simple epithelial hyperplasia (4–7 cell layers) were seen at all intervals. Foci of inverted or exophytic nodular or papillary hyperplasia were seen occasionally at week 3, with frequent mild acute inflammation and loose fibrosis. At week 6, fibrosis and acute or chronic inflammation were present in the lamina propria beneath hyperplastic foci and multifocal nodular or papillary hyperplasia (up to eight cell layers) was evident. The changes were more severe than those seen in concurrent studies with formalin solution. A significant ($p < 0.01$) increase in [^{3}H]thymidine incorporation was seen in comparison with sham-operated controls, which had occasional mild fibrosis in the lamina propria but no hyperplasia. The hyperplastic response was enhanced in the presence of urine, which had been found, in previous studies, to contain tumour-promoting substance(s) (Homma & Oyasu, 1986).

(v) *Acrylic substances (IARC, 1979b, 1986, 1994b)*

Evidence that a biological response to an implant may be more dependent on toxic components leaching from implanted materials than on the properties of the bulk material itself was obtained in a study investigating the effects of subcutaneous implan-

tation of three acrylic denture base resins in guinea-pigs. A specified auto-polymerized pour resin, a heat-polymerized resin and a specified auto-polymerized dough resin were polymerized in 10 mm × 1.5 mm polyethylene tubes one to four days before implantation. They were not sterilized. A total of 20 female guinea-pigs that had served as controls in a maximization test and had been given Freund's complete adjuvant (FCA) received one implant per animal through a 5-mm incision in the back. Two groups of seven animals were observed for 14 or 30 days and one group of six animals was observed for 90 days. A further group of seven implanted animals that had not previously received FCA was observed for 30 days but no difference from the FCA-treated group was detected. An inflammatory response with a fibrous capsule was observed, which decreased with time. The apparent resolution of inflammation at 90 days was most pronounced with the heat-polymerized resin (reaching statistical significance) and least pronounced with the dough polymer. A reduction in width of the capsule was noted. The authors speculated that the gradation in severity of response was partially linked to the irritant properties of residual methyl methacrylate monomer, which was 3% in the dough (which elicited the greatest response) and the pour resin and 1% in the heat-polymerized resin (which was the best tolerated). Other residual chemicals were also suggested as having a role in mediating the response. A link between inflammation and release kinetics of residues or breakdown products was postulated (Kallus, 1984).

(vi) *Ceramics, hydroxylapatite*

Implants of porous and dense hydroxylapatite ($Ca_{10}(PO_4)_6(OH)_2$; pore sizes 100 μm and 3 μm, respectively) were placed in the ears of groups of Wistar rats. Each ear had two implants, either both porous or both dense, inserted into a hole in the bony wall of the middle ear. A total of 116 dense and 120 porous implants were studied in normal ears, and 70 of each in infected ears. Infection was induced three weeks before analysis by intratympanic injection of a *Staphylococcus aureus* suspension in saline. Histopathological examination and electron microscopy up to one year after implantation revealed a fibrotic and epithelial response in the vicinity of the implants, most pronounced in the first month after implantation (assessed by [^{3}H]thymidine incorporation). Covering of the implant was due not only to cell proliferation but also to cell migration over the implant area. The reaction stabilized after three months. There was a granular layer between the bone and the implant, except where inflammatory cells were present. Pores were initially filled with exudate and fibrous tissue, followed by bony infiltration, the greater pore size being associated with a greater amount of infiltration. In the infected ears, the presence of the hydroxylapatite did not affect the inflammatory reactions (oedema, vasodilatation, osteoresorption followed by bone deposition), nor did infection influence the reactions on or in the implant. No other cellular response was noted (Grote *et al.*, 1986).

Inflammatory changes were noted following the implantation of glass–ceramic materials into rabbit ear bones for up to two years. Inflammation subsided within two

months of implantation. Mucous membrane and bone overgrowth of bioactive glass–ceramic was noted. Lysis of the implant as part of bone remodelling (with giant cell infiltration) was seen at one to two years. No changes suggesting a predisposition to neoplasia were evident from this remodelling process (Reck, 1984).

(vii) *Dental composites*

Cell culture studies

The available cell culture techniques are useful as screening tests to compare potential toxicity of dental materials and their components, but not as usage tests, e.g., to predict pulp reactions.

In an in-vitro test system for assessment of the toxicity of dentine bonding agents after penetration through 100-µm or 50-µm slices of dentin (dentin barrier test), three such agents were found to be highly cytotoxic for cultured hamster fibroblasts, in the absence of adequate lining (Meryon & Brook, 1989). In a similar study with human pulpal fibroblasts, cytotoxic effects of bonding materials were also shown following diffusion through dentin. In some cases, such effects were seen even after seven days (Bouillaguet *et al*., 1998).

In a study with freshly extracted or cryo-preserved teeth, simulated pulpal pressure appeared to increase the toxicity of dental bonding agents towards mouse L-929 fibroblasts (Camps *et al*., 1997).

The cytotoxicity of various bonding agents was further demonstrated on the basis of growth of mouse L-cells on microscope slides coated with these materials (Schaller *et al*., 1985).

It has been reported that the toxicity of bonding materials for in-vitro cultured L-929 cells diminished after a few days' pre-incubation of the specimens in culture medium at 37°C, and had disappeared after six weeks of incubation. The authors conclude that bonding agents are unlikely to induce chronic toxicity and that the benefits of preventing microleakage after dental restorations far exceed the potential risk of cytotoxic effects from these materials (Schedle *et al*., 1999).

A resin-based dental filling material containing bis-GMA and TEGMA produced toxic reactions in cultured BALB/c 3T3 cells, which could be eliminated by extraction of leachable components into suitable organic solvents, such as ethanol, chloroform and toluene. The primary active component was identified as unreacted bis-GMA (Rathbun *et al*., 1991).

In an in-vitro test system with human HEp-2 cells, the cytotoxicity of light-cured dental composites was shown to decrease with increasing curing time (Puza *et al*., 1990). Comparable results were found with various dental resin materials tested on human gingival fibroblast cultures (Caughman *et al*., 1991). Similarly, extracts obtained from composite resins cured by either visible or ultraviolet light showed strong toxicity towards human HeLa cells or mouse L-929 fibroblasts in the early stages of extraction, but the effects diminished rapidly and were no longer detectable after six weeks of extraction (Nakamura *et al*., 1985; Schedle *et al*., 1994).

Various types of composite resin were reported to show slight cytotoxicity for an appreciable time after setting (Schedle *et al*., 1999), in sharp contrast to the marked toxicity of the resin monomer itself (Nakamura *et al*., 1985).

Animal studies

The pulpal effects of acid etching in deep cavities have been studied in monkeys. Inflammatory responses and odontoblast displacement were seen three days after etching of cavities with 1% citric acid, but the effects were less severe after 59 days and could be largely suppressed in teeth treated with a liner containing calcium hydroxide after acid application (McInnes-Ledoux *et al.,* 1985).

The pulpal effects of citric acid on surgically exposed root dentin have also been studied in cats. After 21 days, the frequency of adverse pulpal responses to surgery and citric acid treatment is significantly greater than to surgery alone (Ryan *et al.,* 1984).

The pulpal effects of bonding agents have been studied in teeth of ferrets and monkeys filled with composite materials, complicating evaluation of the effects of bonding *per se*. The adverse effects of bonding were reported to be less pronounced in the canine teeth of ferrets than effects of the composite filling material and could be almost eliminated with a calcium hydroxide lining (Plant *et al.,* 1986). In similar studies in monkeys, more pulpal reaction was observed in teeth with composite fillings than in those where a bonding and a composite filling had been placed. The presence of bacteria in the cavities has been suggested to cause some of the effects seen in teeth without bonding agents. With increasing observation time, the degree of pulp inflammation tends to decrease markedly (Hörsted-Bindslev, 1987; Harnirattisai & Hosoda, 1991). Other studies in monkeys have revealed more irritation dentin formation—a sign of initial damage to odontoblasts—in teeth lined with dental adhesives before placement of resin-bonded inlays than in teeth where no adhesives were used (Inokoshi *et al.,* 1995).

A comparison was made of the periodontal effects of silver amalgam and composite restorations placed on the roots of incisor teeth which had been extracted from monkeys and were replanted within an hour after the treatment. Whereas the amalgam produced a localized inflammation that subsided with the formation of a fibrous capsule, the composite resin restorations caused a chronic inflammatory response in the periodontal membrane (Nasjleti *et al*., 1983).

Implantation studies with dental composites have shown persistent inflammation at the implant site after periods of up to three months in rats (Wennberg *et al*., 1983; Steinbrunner *et al*., 1991), ferrets (Grieve *et al*., 1991) and monkeys (Hörsted *et al*., 1986). As bacterial contamination was found in dentinal tubules below the fillings in all these studies, it is difficult to assess which of the effects can be attributed to the composite materials.

New cohesive bonding systems prevent postoperative hypersensitivity and completely seal the tooth–restoration interface, preventing bacterial infection of the underlying substrate and ultimately reducing recurrent caries beneath the hybridized restoration (Cox *et al.*, 1995).

(viii) *Components of dental composites*

The toxicity of materials used in dental composites is summarized in Table 57.

The potential toxicity of the components of resin composites and/or bonding agents has been studied with cell culture techniques. The inhibitory effects of 11 components of resin composites on DNA synthesis, total protein content and protein synthesis of BALB/c 3T3 fibroblasts were investigated. Ethoxylated bisphenol A dimethacrylate was the most toxic compound tested (LC_{50} between 1 and 10 μmol/L). The LC_{50} values for seven other components, namely, bis-GMA, urethane dimethacrylate (UDMA), TEGDMA, 1,6-hexanediol dimethacrylate, glycidyl methacrylate, bisphenol A and bisphenol A diglycidyl ether ranged between 10 and 100 μmol/L, while the LC_{50} values of *N*,*N*-dihydroxyethyl-*p*-toluidine, camphorquinone and *N*,*N*-dimethylaminoethyl methacrylate were above 100 μmol/L. The authors noted that the concentrations to which cells and tissues are actually exposed *in vivo* are not known (Hanks *et al*., 1991).

The toxic interactions of various methacrylic esters used as dentin bonding agents in mouse BALB/c 3T3 fibroblasts *in vitro* were tested by measuring cell survival with the dimethylthiazolium-diphenyl-tetrazolium-bromide (MTT) test. Toxicity of the single compounds increased in the order 2-hydroxyethyl methacrylate<<<TEGDMA <UDMA<bis-GMA. Cytotoxicity also increased with incubation time (Ratanasathien *et al*., 1995).

The rank order of cytotoxicity (ED_{50} values) of composite resin components tested in BALB/c 3T3 fibroblasts was reported to be bis-GMA (0.12 mmol/L) > 2,6-di-*t*-butyl-4-methylphenol (0.16 mmol/L) > triphenyl antimony (0.51 mmol/L) > camphoric anhydride (1.75 mmol/L) > 2-hydroxy-4-methoxybenzophenone (3.54 mmol/L) > dimethyl-*p*-toluidine (3.6 mmol/L) (Lehmann *et al*., 1993).

The cytotoxicity of UDMA in human KB cells was investigated by means of flow cytometric analysis of cellular DNA content. Depending on the concentration of the oligomer, UDMA functions in both a cytostatic (at 10 and 25 μmol/L) and cytotoxic (at 50 μmol/L) manner (Nassiri *et al*., 1994).

The resin components UDMA, bis-GMA, TEGDMA, bisphenol A, glycidyl methacrylate and *N*,*N*-dihydroxyethyl-*p*-toluidine were reported to evoke either immunosuppressive or immunostimulatory activities on the mitogen-driven proliferation *in vitro* of purified T-lymphocytes, activated by accessory cells from the dental pulp, and spleen cells of the rat (Jontell *et al*., 1995).

The components of resin composites are hazardous in so far as all show significant toxicity in direct contact with fibroblasts. However, these components have different cytotoxic potencies and the risk to dental pulp depends upon the quantities which permeate the dentin and accumulate in the pulp (see also Wataha *et al*., 1994).

Blood lipid peroxidation and haemolysis were observed upon irradiation of cultured dog erythrocytes overlaid with a solution of photoinitiators used for light-cured composites. These effects were concentration-dependent (Fujisawa *et al*., 1986).

Table 57. Acute and chronic toxicity of materials used in the composition of dental resins

Compound	In-vitro toxicity	In-vivo toxicity	Mutagenicity/genotoxicity	References
Methacrylic acid	Day 10 rat embryos in culture: positive indices of teratogenicity and cell death at 1.2–2.1 mM	Corrosive to eye and skin	*In vitro*: DNA-binding: positive *S. typhimurium:* negative *In vivo*: no data	Rogers *et al*., 1986 Greim *et al*., 1995
Bisphenol A-glycidyl methacrylate	ID_{50} growth inhibition test: 3T3 mouse fibroblasts: 120 µM Gingiva fibroblasts: 80 µM Growth inhibition of human gingival carcinoma cells: ID_{10} = 10 µg/mL; no-effect conc. 0.6 µg/ml Inhibition of DNA synthesis in BALB/c 3T3 cells: ID_{50} = 13 µM	Irritating	Negative in *Salmonella* umu test No increase in chromosomal aberrations in occupationally exposed workers	Lehmann *et al*., 1993 Leyhausen *et al*., 1995 Hanks *et al*., 1991 Mitelman *et al*., 1980 Imai *et al*., 1988
Bisphenol-A dimethacrylate	Oestrogenic activity in MCF7 cells (1/10 000 of oestradiol)	No data	No data	Olea *et al*., 1996
Ethoxylated bisphenol-A dimethacrylate	Inhibition of DNA synthesis in BALB/c 3T3 cells: ID_{50} = 3.3 µM	No data	No data	Hanks *et al*., 1991
Triethylene glycol dimethacrylate	Inhibition of DNA synthesis in BALB/c 3T3 cells: ID_{50} = 70 µM Growth inhibition of rat osteoblasts: IC_{50} = 700 µM Stimulation of mitogen-driven T-lymphocyte proliferation	No data	*S. typhimurium* negative, 5 strains, ± S9 (up to 10 000 µg/plate) Positive in V79/HPRT gene mutation assay and in micronucleus assay in V79B cells	Suda & Kawase, 1991 Jontell *et al*., 1995 Hanks *et al*., 1991 Schweikl *et al*., 1998 Schweikl & Schmalz, 1999
Ethylene glycol dimethacrylate	Moderate reaction with cellular glutathione (EC_{50} = 1.3 mM)	No data	No data	McCarthy *et al*., 1994
Diethylene glycol dimethacrylate	No data	No data	*S. typhimurium* negative, 5 strains, ± S9, 40–2500 µg/plate	Waegemaekers & Bensink, 1984
Tetraethylene glycol dimethacrylate	Reduced survival of mouse lymphoma L5178 cells at 350–525 µg/mL	No data	Positive in mouse lymphoma L5178 *tk*-locus mutation assay, micronucleus test and chromosomal aberration test in L5178 cells, at 350–525 µg/mL	Dearfield *et al*., 1989

Table 57 (contd)

Compound	In-vitro toxicity	In-vivo toxicity	Mutagenicity/genotoxicity	References
Urethane dimethacrylate	Growth inhibition of human gingival carcinoma cells: ID_{10} = 9 µg/mL; no-effect conc. 0.6 µg/mL; Inhibition of DNA synthesis in BALB/c 3T3 cells: ID_{50} = 11 µM Stimulation of mitogen-driven T-lymphocyte proliferation	No data	No data	Imai *et al.*, 1988 Hanks *et al.*, 1991 Jontell *et al.*, 1995
1,4-Butanediol dimethylacrylate	No data	No data	*S. typhimurium* negative, 5 strains, ± S9, 40–2500 µg/plate	Waegemaekers & Bensink, 1984
1,6-Hexanediol dimethacrylate	No data	No data	*S. typhimurium* negative, 4 strains, ± S9, 40–2500 µg/plate	Waegemaekers & Bensink, 1984
2-Hydroxyethyl methacrylate	Growth inhibition of human gingival carcinoma cells: ID_{10} = 250 µg/mL; no-effect conc. 30 µg/mL Human gingival cells, inhibition of cell growth rate to 24% of control	No data	*S. typhimurium* negative, 4 strains, ± S9, 40–2500 µg/plate	Imai *et al.*, 1988, 1992 Waegemaekers & Bensink, 1984
Methyl methacrylate	Growth inhibition of human gingival carcinoma cells: ID_{10} = 1000 µg/mL; no-effect conc. 100 µg/mL	Release from poly(methyl methacrylate) orally from palatal appliances in humans, local concentration: 180 µg/mL; release rate 29 µg in the first hour (non-toxic) Dermatitis, eczema and sensitization can be adverse reactions in dentists and dental technicians Allergic sensitization also observed in dental patients	*S. typhimurium* negative, 5 strains ± S9, 40–10 000 µg/plate Positive in mouse lymphoma L5178 *tk*-locus mutation assay, micronucleus and chromosomal aberration assay in L5178 cells, and in induction of chromosomal aberrations in rat bone marrow *in vivo*	Imai *et al.*, 1988 Waegemaekers & Bensink, 1984 Baker *et al.*, 1988 IARC, 1994b Fisher, 1982 Seppäläinen & Rajaniemi, 1984 Donaghy *et al.*, 1991
Benzoyl peroxide	Induction of DNA strand breakage in mouse keratinocytes	Skin tumour promoter in mice	*S. typhimurium* negative, 7 strains ± S9	Hartley *et al.*, 1987 IARC, 1999b

Table 57 (contd)

Compound	In-vitro toxicity	In-vivo toxicity	Mutagenicity/genotoxicity	References
Bisphenol A	Oestrogenic activity in MCF7 cells (1/10 000 of oestradiol). Inhibition of DNA synthesis in BALB/c 3T3 cells: ID_{50} = 30 µM	No developmental toxicity at maternal toxic (rats) or lethal (mice) dose levels	*In vivo* DNA-adduct formation in livers of male rats	Olea *et al.*, 1996 Hanks *et al.*, 1991 Morrissey *et al.*, 1987 Atkinson & Roy, 1995
2-Hydroxy-4-methoxy-benzophenone	No data	Common ingredient of sunscreens and tanning agents; may cause contact dermatitis in sensitive individuals. No irritation or inflammation in human pulpa. Depigmentation of the skin in occupationally exposed persons. No irritation in the rabbit eye.	*S. typhimurium* negative, 5 strains ± S9 *In vitro*: *S. typhimurium* weakly positive with S9 Induction of sister chromatid exchange and chromosomal aberrations *In vivo*: no genotoxicity in mice Positive in mouse lymphoma test at 22–52 µg/mL	Bonin *et al.*, 1982 National Technical Program, 1992 Stanley *et al.*, 1979
Camphorquinone	Inhibition of DNA synthesis in HeLa cells in the cytotoxic range	No data	*S. typhimurium* negative, TA 100 ± S9, up to 25 µmol/plate *S. typhimurium* negative, in 5 strains ± S9 up to 10 µmol/plate	Leyhausen *et al.*, 1995 Dorado *et al*., 1992 Cameron, 1993
p-Methoxyphenol	No data	Depigmentation of the skin of occupationally exposed workers. Dietary admin. in mice at 0.1%, slight growth depression; in rabbits at 10% little or no growth depression; in dogs fed 6 g/day, no deleterious effects. Undiluted application (> 1 day) on rabbit eye or skin causes damage and necrosis	*S. typhimurium* negative, 5 strains, ± S9	Chivers, 1972 Clayton & Clayton, 1993

Table 57 (contd)

Compound	In-vitro toxicity	In-vivo toxicity	Mutagenicity/genotoxicity	References
N,*N*-Dimethyl-*p*-toluidine	No data	No data	*S. typhimurium* negative, 3 strains ± S9 *S. typhimurium* positive, 1 strain ± S9 Positive in mouse lymphoma test and in micronucleus test in V79 cells Induction of DNA breakage *in vivo* in mouse and rat liver	Taningher *et al*., 1993 Cameron, 1993

ID_{50} or IC_{50} = concentration inhibiting the effect by 50%; S9 = liver homogenate preparations for metabolic activation
(Modified from Schedle *et al*., 1999)

The metabolism of camphorquinone (used as a composite initiator) was studied in rabbits following oral administration of 500–800 mg of the compound. Although 40% of the dose was excreted as the glucuronide in urine, camphorquinone did not possess any pharmacological activity (Robertson & Hussain, 1969).

In a study on embryotoxicity in three-day chicken embryos, benzoyl peroxide caused malformations at moderate frequency (Korhonen *et al.,* 1984).

Treatment with 200 mg/kg bisphenol A resulted in DNA-adduct formation in rat liver, demonstrated by ^{32}P-postlabelling (Atkinson & Roy, 1995). Daily oral treatments of pregnant rats and mice with bisphenol A at doses of 160, 320 and 640 mg/kg bw (for rats) and 500, 750, 1000 and 1250 mg/kg bw (for mice) during gestational days 6–15 caused reduced maternal body weight gain in the rats and up to 18% maternal mortality in the mice, at the high dose. The percentage of resorptions per litter was significantly increased in mice treated with the 1250-mg/kg dose. There was no significant effect of bisphenol A treatment on any of the parameters of developmental toxicity in either species, even at doses that caused significant maternal toxicity or mortality (Morrissey *et al*., 1987). It appears unlikely that bisphenol A produces toxic responses *in vivo* through its weak oestrogenic activity (Olea *et al*., 1996).

(ix) *Other materials*

In an assessment of hernia repair meshes made of three types of polypropylene implanted in the abdominal wall of rabbits, histological examination after up to 90 days revealed the accumulation of macrophages and the appearance of connective tissue and white adipose tissue. The tissue response depended on the presence of an intact parietal peritoneum (Bellon *et al*., 1998).

Various forms of artificially degraded poly(L-lactide) or undegraded low-molecular-weight poly-L-lactide displayed minimal differences in their effects on a mouse macrophage cell line (IC21). Parameters studied included cell number, lactate dehydrogenase, prostaglandin E_2 and morphology. Latex beads and Zymosan were used as controls. The authors concluded that degradation of poly-L-lactide had no effect on its toxicity (Dawes & Rushton, 1997).

(*b*) *Immunological effects*

(i) *Polydimethylsiloxanes (silicones)*

Concern over the possibility of immune disease arising from the use of silicone gel breast implants stimulated experimental studies of the immunotoxicity of silicones. Extensive analysis of the immunotoxic potential of silicones has failed to reveal any factor that would be likely to promote carcinogenesis. Animal studies have shown that silicones are not significantly immunotoxic but that some can act as adjuvants under experimental conditions; however, they have little potential to elicit specific antibody responses or cell-mediated immunity.

The immunotoxicity of subcutaneously administered materials, including silicone fluid (Dow Corning 360), gel and elastomer (from Silastic breast implants) and poly-

urethane in female B6C3F_1 mice was investigated over 10 and 180 days. A number of positive controls were used to confirm that the assays were able to detect the relevant immunotoxicity parameters. None of the following parameters were significantly altered in silicone-treated mice: body weight, organ weight, haematology, blood chemistry (alanine aminotransferase, urea, glucose, albumin, total protein, serum CH 50 and C3 levels), cellularity of bone marrow and cerebrospinal fluid, antibody response to sheep erythrocytes, proliferative response to concanavalin A, phytohaemagglutinin, lipopolysaccharide and allogenic cells, reticuloendothelial function, serum complement, host resistance to *Streptococcus pneumoniae* and B16F10 tumour cells, distribution of splenic B and T cells, and natural killer cell activity after 10 days. A modest protection to challenge with *Listeria monocytogenes* was observed with all silicones and there was some evidence of inflammatory activity with silicone gel. After 180 days, all the materials tested marginally reduced the level of immunoglobulin-positive cells in the spleen but had no consistent effect on the distribution of T-cell surface markers. A modest depression of natural killer cell activity was observed after 180 days in all silicone-treated groups. In further studies to investigate the dose–response relationships of this effect, the reduction of natural killer cell activity was confirmed following exposure to silicone gel, but not with silicone elastomer. These relatively minor changes in natural killer cell activity, in the absence of any changes in the other parameters tested, indicate that there were no significant immunotoxic effects (Luster, 1993; Bradley *et al*., 1994a,b).

A number of experimental studies in rats have addressed the ability of silicones to act as immunostimulatory agents or adjuvants. In one study, silicone oil was reported to be a weak adjuvant, while silicone gel showed a strong adjuvant effect (Naim *et al*., 1993). In contrast, in another study, only weak enhancement of a specific immune response was found, and silicones failed to act as non-specific immunostimulants (Chang, 1993).

None of these findings are indicative of immunotoxic effects that might influence carcinogenicity.

(ii) *Other materials*

An inhibitory effect on immunocompetence was noted in poorly reported studies involving the implantation of various materials into mice. Groups of female C57BL/6 mice (total, 380) were given implants of discs (15 × 2 mm) of poly(ether urethane), silicone elastomer, poly(methyl methacrylate) and an amorphous-phase calcium phosphate ceramic (bioglass) into subcutaneous dorsal pockets. At the same time, tumours were induced by injection of suspensions of 3-methylcholanthrene-induced mouse fibrosarcoma cells, which are known to produce tumours within 28 days. The tumour cell suspension was injected over and around the implant. Controls received tumour cells without implants. Splenic lymphocytes were harvested at 28 days to study the effect of the polymers on immunocompetence. In assays with the mitogens lipopolysaccharide and phytohaemagglutinin, inhibition of lymphoproliferation was caused by

the polyurethane and poly(methyl methacrylate), while bioglass and silicone had no effect. Sensitization to M_4 tumour antigen was depressed in lymphocytes from mice implanted with polyurethane, silicone or the ceramic (Habal *et al*., 1980).

5B.3 Genetic and related effects

5B.3.1 *Humans*

Clastogenicity was clearly associated with worn orthopaedic implants in a limited study of 71 revision arthroplasty patients and 30 primary arthroplasty controls (Case *et al*., 1996). This study is described in Section 5A.4.1.

5B.3.2 *Experimental systems*

As in the case of metallic devices, non-metallic devices have been tested mainly by the use of extracts. Certain materials used in the preparation of cements have been previously evaluated in *IARC Monographs*: acrylic acid (IARC, 1979c), methyl methacrylate (IARC, 1994b), methyl acrylate and ethyl acrylate (IARC, 1986).

(*a*) *In-vitro genotoxicity assays*

The majority of the studies have been performed with dental materials.

Twenty-seven dental materials were tested in a battery of three in-vitro assays: the bacterial *umu*-test, the eukaryotic DNA synthesis inhibition test in HeLa cells and the alkaline filter elution technique for detection of strand breaks in the DNA of gills taken from exposed clams. Some dental materials were tested as single substances present in dental devices (monomers, inhibitors, co-initiators or photo-initiators) or as extracts of materials. Frequently the materials consisted of about 10–20 single ingredients (root canal filling material, composites or ionomer cements). The dental materials were extracted in serum, culture medium or dimethyl sulfoxide (DMSO) for 24 h at 37°C. The extracts of two widely used root-filling dental materials (Vitrebond® and AH26®) produced dose-dependent effects in all three test systems (Heil *et al*., 1996).

The mutagenicity observed in various assays with the epoxy resin sealer AH26 was attributed to formaldehyde released from decomposition of hexamethylene tetramine, a component of this endodontic material (Geurtsen & Leyhausen, 1997). A DMSO extract of AH26 was reported to be mutagenic in *Salmonella typhimurium* strain TA100 and its epoxy-bisphenol A resin component was associated with the mutagenic properties of this sealer (Ørstavik & Hongslo, 1985). Extracts of the dental material N2®, which contains paraformaldehyde, showed genotoxic activity in the bacterial *umu*-test (Heil *et al*., 1996). Several of the substances present in some dental materials demonstrated a mutagenic effect when tested separately, but extracts of dental materials that contain mutagenic components did not always show mutagenicity.

Two of six orthodontic direct-bonding resin systems tested were shown to be mutagenic in *S. typhimurium* strain TA100 both before and after curing using aqueous and DMSO extracts. No metabolic activation was necessary. The mutagenic agent(s)

were not chemically identified, but when the formulation of one of the mutagenic bonding resins was changed by the manufacturer, the product was no longer mutagenic (Cross *et al.*, 1983).

The mutagenic potential of saline and DMSO extracts of another dental adhesive, Syntac®, was demonstrated in *S. typhimurium* strains TA102 and TA104. Glutaraldehyde, an ingredient of this type of adhesive, was mutagenic in these same strains. In the same study and using the same protocol, two other dentin-bonding agents, Pertac® and Prisma® Universal Bound, did not show mutagenic activity (Schweikl *et al*., 1994). A physiological saline extract of Prisma Universal Bond 3 adhesive, which contains glutaraldehyde, was strongly mutagenic in *S. typhimurium* TA102 (Schweikl *et al*., 1996).

Compounds of commercially available dental material kits were tested for mutagenicity in *S. typhimurium*. One of these materials, Gluma 3, contains glutaraldehyde and 2-hydroxyethyl methacrylate and was highly mutagenic in strains TA100 and TA104 (Li *et al*., 1990). DMSO extracts of Gluma 3 and two other glutaraldehyde-containing dentin-bonding agents, Syntac adhesive and Prisma Universal Bond 3 adhesive, were reported to cause mutations in the *hprt* locus of Chinese hamster lung V79 cells (Schweikl & Schmalz, 1997).

Components of three glass ionomer cements were mixed and allowed to polymerize for one hour or one week. Extracts were prepared by incubation of 1 g of the material in 5 mL phosphate-buffered saline for three days at 37°C. The extracts were tested for induction of sister chromatid exchanges in human lymphocytes *in vitro*, in the presence or absence of a metabolic activation system (microsomal S9 fraction). One of the extracts obtained one hour after polymerization caused induction of sister chromatid exchanges, while the other two showed weak or no activity. The extract obtained from the first material after one week showed activity, while those from the two other preparations were inactive in inducing sister chromatid exchanges. In general, the activity of the extracts in this assay was higher in the absence of S9 than in its presence. The composition of the dental cements was not given (Stea *et al.*, 1998).

Non-shrinking dental epoxy-copolymers, containing spiroorthocarbonates and various epoxy derivatives, were tested for mutagenicity in *S. typhimurium*. The weak mutagenic activity found with strain TA97a was attributed to the epoxy formulation rather than to the spiroorthocarbonate component (Yourtee *et al.*, 1994).

Saline (0.9% sodium chloride) extracts of thermoplastic polyurethanes, used as insulating materials for cardiac pacemaker leads, did not show mutagenic activity in *S. typhimurium* (Pande, 1983).

Methanolic extracts of three segmented polyurethanes, one non-segmented polyurethane and silicone containing 25% silica were tested for induction of chromosomal aberrations in Chinese hamster lung cells. No activity was found (Nakamura *et al*., 1992).

Alumina ceramic and ultra-high molecular weight polyethylene are used in the manufacture of pivot bearings in centrifugal blood pumps for cardiopulmonary

bypass. Extracts were prepared by incubation of these materials in saline for 72 h at 50°C, and tested for mutagenicity in *S. typhimurium* strains TA97a, TA98, TA100, TA102 and TA1535, with or without metabolic activation. No mutagenicity was detected (Takami *et al*., 1997).

A bioresorbable membrane (Soprafilm), made of hyaluronic acid and carboxymethyl cellulose, was tested for mutagenic activity in *S. typhimurium* using extracts in saline, DMSO and saline/ethanol. No mutagenic activity was detected (Burns *et al*., 1997). [The Working Group noted that the method used to prepare the extract and the conditions of testing were not clearly presented.]

Kevlar®49 (a poly-*para*-phenylene-terephthalimide) was tested for mutagenic activity. Test samples were the raw material (ground powder), and extracts in chloroform or ethanol. None of the samples showed mutagenic activity in the six *S. typhimurium* strains tested. In a mammalian mutagenicity assay, Kevlar®49 extracts obtained by incubation of the materal in cell culture medium or in DMSO for seven days at 37°C, did not show mutagenicity at the *hprt* locus in Chinese hamster lung V79 cells (Wening *et al*., 1995).

A bone cement containing methyl methacrylate, *N*,*N*-dimethyl-*para*-toluidine and hydroquinone (Surgical simplex P) was tested in an in-vitro micronucleus assay with human lymphocytes from 15 different donors. Clearly positive results were demonstrated with freshly polymerized cement and with cement that had been preincubated in cell culture medium for five days before the assay (Bigatti *et al*., 1994). The same material was reported to be inactive in an assay to determine sister chromatid exchanges in cultured human lymphocytes, but in those experiments a significant decrease in the cell proliferation index was observed (Bigatti *et al*., 1989).

In a DNA synthesis inhibition test with HeLa cells, the effects of bis-GMA were masked by the cytotoxicity of the chemical, although no mutagenic activity was observed in the bacterial *umu*-microtest (Leyhausen *et al.,* 1995). A total of 27 acrylate esters, among which 1,4-butanediol dimethacrylate, diethylene glycol dimethacrylate and 1,6-hexanediol dimethacrylate were reported to be non-mutagenic in *S. typhimurium* when tested with five strains with and without metabolic activation (Waegemaekers & Bensink 1984). Tetraethylene glycol dimethacrylate, identified as an impurity in composite dental materials, was found to be genotoxic in several *in vitro* assays: the micronucleus test, the chromosomal aberration test and the mouse lymphoma mutagenicity test with cultured L5178Y cells (Dearfield *et al.,* 1989).

The genetic effects of benzoyl peroxide, used as a polymerization initiator, have recently been reviewed (IARC, 1999b).

The bone cement polymerization accelerator *N*,*N*-dimethyl-*para*-toluidine was weakly mutagenic (Miller *et al.,* 1986) or non-mutagenic in *S. typhimurium* strains TA97, TA98 and TA100 with rat and hamster S9 (Taningher *et al.,* 1993; Cameron, 1993). However, this compound was positive in the mouse lymphoma test (Cameron, 1993), induced micronuclei *in vitro* in V79 hamster cells with an increase of non-disjunction (at doses of 0.9 and 1.9 mM), and gave rise to DNA damage *in vivo* in

mouse liver (at 1 mmol/kg i.p.) and rat liver (at 4 and 8 mmol/kg i.p. and 8 mmol p.o.) as determined in the alkaline elution assay (Taningher *et al*., 1993). The photosensitizer camphorquinone was non-mutagenic in *S. typhimurium* TA 100 strain and in a battery of different bacterial strains with or without activating systems (Cameron, 1993). Inhibition of DNA synthesis in HeLa cells by camphorquinone was masked by the cytotoxicity of the compound (Leyhausen *et al.,* 1995). The inhibitor *p*-methoxyphenol was non-mutagenic in different strains of *S. typhimurium* (Haworth *et al.,* 1983). The UV stabilizer 2-hydroxy-4-methoxybenzophenone did not show mutagenic activity in five strains of *S. typhimurium* with or without rat S9 (Bonin *et al.,* 1982). These results were later confirmed by Zeiger *et al.* (1987). The dental composite additive triphenyl antimony inhibited DNA synthesis in HeLa cells but had no mutagenic activity in the bacterial *umu*-microtest (Leyhausen *et al.,* 1995).

N-Acryloyl-*N*′-phenylpiperazine (Acr NPP) is a promoter of redox reactions that has been proposed as a polymerization activator of acrylic resins for biomedical use. It was tested in a battery of genotoxicity tests. No mutagenic activity was detected in three strains of *S. typhimurium* (TA97, TA98 and TA100) with or without metabolic activation. However, because of bacterial toxicity, the highest level tested was only 0.3 μmol/plate. In the DNA damage–alkaline elution test with hamster V79 cells treated in culture for 24 h with 10 mM of this compound and with liver cells isolated from mice 24 h after in-vivo treatment by i.p. injection with 1 mmol/kg bw, a weak but statistically significant increase in DNA fragmentation was observed, but this effect was associated with a high level of cytotoxicity. In the micronucleus test with cultured V79 hamster cells, Acr NPP showed a dose-dependent effect that reached about 25-fold the level of the background controls. Immunofluorescent staining with antibodies against kinetochore proteins revealed that micronuclei were due to an aneugenic mechanism and not to a clastogenic effect, as confirmed by the alkaline DNA elution test. Acr NPP is to be regarded as an aneugen (Taningher *et al.*, 1992).

Liu *et al*. (1997) tested a nonceramic hydroxylapatite, used as a calcium phosphate cement, for gene mutation in *S. typhimurium* TA98 and TA100, for unscheduled DNA synthesis in Chinese hamster ovary cells using a scintillation method and in a mouse bone marrow micronucleus induction assay. Crushed and ground specimens were extracted with physiological saline (1:2, w:v) for 24 h at 37°C. There was no determination of the chemical composition of the extracts or of their physicochemical properties (pH, osmolality). No effects were demonstrated in any of the assays, but there was no evidence that anything had been extracted from the material.

(*b*) *Cell transformation test*

Cells growing *in vitro* from explants of subcutaneous connective tissue from adult BALB/c mice were grown for 18 or more days before being implanted while attached to a plastic plate. Tumours formed after 24–79 weeks, the latent period before tumour appearance being correlated inversely with the time period during which the cells were cultured *in vitro*. Tumours appeared to be composed of histologically undifferentiated

sarcoma cells that were transplantable without plates. When inoculated in saline suspension, the cells did not form tumours until they had been in tissue culture for 12 weeks. The plastic plates alone did not induce tumour formation within more than 1.5 years of implantation. The authors concluded that a smooth surface was acting as a carcinogen first *in vitro* and then *in vivo* and that factors related to the geometry and mechanics of cell attachment to a flat surface play a role in this process (Boone *et al*., 1979). A BALB/c 3T3 cell transformation assay gave positive results when the cells were cultured with glass dishes coated with poly(ether urethanes) in the presence of *O*-tetradecanoylphorbol 13-acetate (TPA) (Tsuchiya *et al.*, 1996).

(*c*) *In-vivo genotoxicity assays*

Diaminotoluenes have been studied because of their industrial use as intermediates in polyurethane synthesis and because of their potential release by degradation from the poly(ester urethane) covering of some breast implants (see Section 5B.1.1(*a*)). The extent of DNA damage induced by these compounds was determined in female Fischer 344 rats fed 10, 40, 80 or 180 ppm 2,4-diaminotoluene, a carcinogenic isomer, for up to six weeks, or in rats receiving subcutaneous implants of poly(ester urethane) foam (67 or 267 mg/kg) in mammary fat pad. DNA adducts and mutations at the *hprt* gene were determined in spleen T-lymphocytes of rats fed 40 or 80 ppm of 2,4-diaminotoluene for six weeks. No increase in either marker was observed at 1, 6, 12, 20, 28 and 42 weeks after the start of feeding. In the liver and mammary glands, a single major DNA adduct was detected in animals fed 10–180 ppm 2,4-diaminotoluene, while two minor DNA adducts were occasionally observed. DNA adduct levels in the liver reached a plateau three to six weeks after the start of feeding. A similar plateau with DNA adduct levels generally 1.5–4-fold lower than those observed in the liver was seen in the mammary gland. The major DNA adduct was present in the mammary gland up to 43 weeks after implantation. Implantation of poly(ester urethane) foam did not increase DNA adducts in liver, mammary gland or spleen or mutations at the *hprt* gene in spleen T-lymphocytes at the same sampling times (Delclos *et al.*, 1996).

In an abstract, results were summarized of three in-vivo tests, the mouse bone marrow micronucleus assay, the mouse sperm morphology test and the Chinese hamster bone marrow sister chromatid exchange assay. Three uncured dental materials were tested in mice by addition at 50 ppm in the drinking-water for 14 weeks. [The basis for this dose selection was not stated.] One preparation ('Right-On Adhesive') induced sister chromatid exchanges (Dunipace *et al*., 1990).

Saline extracts of two bone waxes were given to Swiss albino mice on two consecutive days via the intraperitoneal route at doses of up to 50 mL/kg bw per day. The mouse bone marrow micronucleus test, performed 24 or 36 h after the second dosing, revealed no genotoxic effect with either extract (Mohanan & Rathinam, 1996). [The Working Group noted that no information about the composition of the waxes or the extracts was provided.]

(*d*) *Cytogenetic effects in tumour cells*

A cytogenetic analysis was performed of cells from implantation site sarcomas from CBA/H and CBA/H-T6 mice implanted with double films of unplasticized vinyl chloride–vinyl acetate copolymer. The principal findings were numerical chromosomal abnormalities in preneoplastic lesions during the early foreign-body reaction and structural abnormalities of specific chromosomes found as stable cell markers during late preneoplasia (Rachko & Brand, 1983).

5B.4 Mechanistic studies of implantation-site sarcomagenesis in rodents

5B.4.1 *Major factors that affect tumour incidence in solid-state carcinogenesis*

Foreign-body carcinogenesis in rodent species has, for many years, been recognized as a classic model of multistage endogenous tumorigenesis that requires half to two-thirds of the lifespan for tumour development. A number of studies have demonstrated that physical and not chemical characteristics are responsible for this phenomenon and that a dose–response relationship is evident with respect to implant size and tumour frequency (Schoen, 1987). The major physical factors that affect the occurrence of tumours in response to foreign bodies in rodents are discussed extensively in Section 4B.22.1.

5B.4.2 *Biological factors*

(*a*) *Fibrous tissue capsule formation and continued presence of implant*

There has been a general consensus that the process of foreign-body tumorigenesis requires two sequential preconditions: (*a*) fibrous tissue capsule formation in linear circumferential fashion; and (*b*) continued presence of the implant in the capsule during the latent period (Oppenheimer *et al*., 1961; Brand *et al*., 1975c; Schoen, 1987; Brand, 1994).

Polystyrene films (15 × 15 × 0.01 mm) were implanted subcutaneously into rats. During each month thereafter, film and surrounding capsule or film only were removed. Three groups of animals were observed: Group 1, rats that retained both film and capsule; Group 2, rats with capsules but no films; and Group 3, rats with neither capsules nor films. Six per cent of the animals in Group 1 developed tumours; no tumours were found in Group 2 when the films were removed before six months, while 11% of the animals developed tumours when the films were removed after six months; no tumour development was found in Group 3 independent of the time of removal of film and capsule (Oppenheimer *et al*., 1958).

The same experiments were carried out with cellophane films. As with polystyrene, no tumours were induced when the capsule was removed as well as the film. However, when films alone were removed during the early months, there was a marked reduction in the number of tumours, although a difference was observed between cellophane and polystyrene: with polystyrene, removal of the film within six months entirely precluded tumour formation, whereas with cellophane, five tumours (8.6%) arose when the film was removed during the first six months (Oppenheimer *et al*., 1964).

In another experiment, when a glass cover-slip implanted on the right side in rats was removed from the tissue capsule after four months, no sarcomas developed, whereas six sarcomas occurred on the left side in association with glass cover-slips that were left in place (Oppenheimer *et al*., 1961).

The effect of film removal was also observed after subcutaneous implantation of non-perforated poly(vinyl chloride) films (22 × 15 × 0.2 mm) in mice. At twelve months after implantation, the number of sarcomas per number of surviving animals was 17/30 (56.6%) when the film was left in place, and 0/20 (0%) and 1/29 (3.6%) when the film was removed after 3.5 months or 6.5 months, respectively (Moizhess & Vasiliev, 1989).

(*b*) *The role of perforation in the reduction of tumorigenicity*

Series of Millipore filter disks with eight different pore sizes were subcutaneously implanted in mice. One, three, five and 10 months after implantation, histological and ultrastructural studies were carried out on specimens composed of the implants and the surrounding tissue. Filters with pore sizes equal to and larger than 0.22 μm were non-tumorigenic and induced tissue reactions characterized by invasion of filters by macrophages and by presence of phagolysosomes within macrophages as evidence of phagocytic activity. In tissue reactions induced by tumorigenic filters, which had pore sizes of less than 0.22 μm, these features were always missing; instead, there were more fibrous capsules (Karp *et al*., 1973).

Similar marked histological differences in the long-term tissue reactions were observed between flat and foamed polyurethane films (Nakamura *et al*., 1995).

(*c*) *Species and strain differences*

The results of several studies suggest that species and strains of animals differ in susceptibility to foreign-body sarcoma development. However, the data on species other than mice and rats are limited.

Poly(methyl methacrylate) discs were implanted intramuscularly into rats and guinea-pigs. Tumours were observed in rats in association with large and medium-sized discs, whereas no tumours were found in guinea-pigs. In the latter species, a considerable decrease in the thickness of the capsule surrounding the implant was observed during the course of the 30-month experiment (Stinson, 1964).

Following subcutaneous implantation of poly(2-hydroxyethyl methacrylate) films, five tumours developed in 10 rats that survived for more than 12 months, whereas no tumours were found in hamsters or guinea-pigs. The occurrence of tumours was associated with the presence of a thick capsule surrounding the implant (Imai & Masuhara, 1982). [The Working Group noted the small numbers of animals.]

Striking differences in tumour response to subcutaneous implantation of vinyl chloride–vinyl acetate copolymer films were found among 18 strains of mice: the incidence of tumours was 90–100% in CBA/H and CBA/H-T6 females, AKR/J males, BALB/cJ and BALB/c Wat females, C57BL/10ScSn females and (C57BL/10ScSc

× CBA/H)F_1 males and females, whereas no tumour was induced in males of strain I/LnJ or strain SJL/J. The incidence in other strains was intermediate (Brand *et al*., 1977).

5B.4.3 *Timing and location of preneoplastic events*

The process of foreign-body tumorigenesis was extensively studied by Brand and coworkers using the techniques of transplantation of fibrous capsules and/or explanted films from strain CBA/H mice into the co-isogenic strain, CBA/H-T6. The presence or absence of the T6 marker chromosome was used to distinguish CBA/H-T6 from CBA/H cells (Brand *et al*., 1967a,b, 1971, 1973, 1975a,b,c; Thomassen *et al*., 1975; Brand, 1976).

Groups of implant-carrying mice were treated every two weeks or monthly as follows: film implants (double pieces of 15 × 22-mm vinyl chloride–vinyl acetate co-polymer) and two thirds of the surrounding tissue capsules were removed and cut into 7 × 15-mm pieces. (*a*) One film pair and the corresponding tissue were separately implanted into fresh recipient animals, either at different sites (left and right flank) of the same animal or into different recipients; (*b*) another third of the film pair was reimplanted into the capsule pocket that had been left in the original carrier; (*c*) the last third of the film and tissue capsule was examined by karyological, histological and in-vitro culture methods.

The results of these experiments were summarized as four main findings. (i) If the capsule or implanted film was transferred after a latent period lasting two to nine months, tumours developed from transplanted film pieces only, not from capsule tissue. Capsule transfer was tumorigenic only in the latest phase of premalignancy, i.e. about four weeks before tumour development in the original animals. (ii) Tumours in the original and corresponding recipient animals were identical, especially in chromosome number, but occasionally also with regard to ploidy level. (iii) Tumours appeared in the original and corresponding recipient animals up to nine months after transfer at almost exactly the same time (within two weeks). (iv) Tumours developed in animals always on one side, either right or left. No independent multiple tumours on both sides were ever recorded. Furthermore, tumours developed regularly from one side of the implant only, either the upper or the lower surface. Film transfer experiments showed that tumours in the original and corresponding recipient animals always developed from the same side of the film fragments. Thus, it was evident that the premalignant cell clone was restricted to only one side of the film.

From these results, it was concluded that: (1) premalignant cells can first be demonstrated five to six months after initial implantation of the plastic film; (2) premalignant cells are firmly attached to the plastic implant at least up to nine months before tumour appearance; (3) premalignant cells are on the film surface in multiple foci, since film cuttings carry equal tumorigenicity; (4) there seems to be no cell division among the film-attached premalignant population; (5) the capsule tissue is free of transplantable premalignant cells until about one month before tumour development; (6) since the tumours in the original and corresponding recipient animals

appear at the same time and are composed karyologically of the same stemline, a specific cell clone must reside on the film at transfer; (7) individual premalignant clones must also exist, because premalignant cells are found only at one implant site and only on one side of the implant; and (8) the specific and stable individuality of the premalignant cell clone suggests that the parent cell may have been already endowed with the clonal characteristics of karyotype and premalignant determination (Brand *et al*., 1967a,b).

A different paper reported that some animals of the 8.5- and 9.5-month transfer groups developed late tumours from tissue capsules remaining after the implants were removed. These late tumours were always heterologous to those developing from corresponding film segments transferred to recipient animals (Thomassen *et al*., 1975).

A more precise transplantation study was carried out to clarify the timing and location of preneoplastic events: the implants and mouse strains used were the same as above, and two main experimental designs were adopted. In the first (Experiment 1), whole unopened film/capsule complexes were exchanged at one, two, three, four or five months after implantation, between animals with and without the T6 marker chromosome; in the second (Experiment 2) only the implanted films were exchanged after 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5 or 6.0 months, between pairs of animals with and without the T6 marker; capsules remained *in situ* but were opened to remove and replace the films.

The results of Experiment 1 showed that, within one month after implantation, transferable preneoplastic cells appeared at the site of the foreign-body reaction in 33% of the animals, and the rate increased rapidly with time to over 70%. In the two-month transfer groups, as well as in later transfer groups, several instances of karyologically identical 'homologous tumours' (descending from a common parent cell) were recorded in recipient and original animals. This was considered to be unequivocal evidence that, before the time of transfer, not only were preneoplastic cells present in the original implant carrier but the parent cell had already expanded into a clone.

Early opening and cutting of capsule and film disturb the conditions to the extent that preneoplastic cells at that stage have little chance of surviving the transfer operation or establishing themselves in the new host where a fresh foreign-body reaction occurs. However, in a few instances (1/4) in Experiment 2, exchange of films between unexcised capsules of different animals was successful. This suggests that preneoplastic cells may settle on implant surfaces at least within 2.5 months after implantation (Brand *et al*., 1971).

The following conclusions were drawn from these results. Tumours arose from cells with the same neoplastic specificity, which indicated their clonal nature. Specific tumour properties must have been predetermined and fixed in these clonal cells before the implants were cut and transferred. The demonstration of such clones implied the prior existence of 'parent cells' in which the initial determining event had already taken place (Brand, 1976).

In order to determine how many preneoplastic parent cells are induced by film implants of a given size, the time-to-tumour data following subcutaneous implantation of vinyl chloride–vinyl acetate copolymer films (7 × 15 and 15 × 22 mm) into CBA mice were analysed statistically. Animals were declared 'tumour-negative' when > 30 months without tumour development elapsed before death. It was calculated that the most probable number of 'preneoplastic parent cells' must be 1 in response to a 7 × 15-mm implant and 3 in response to a 15 × 22-mm implant (Brand *et al*., 1973). These values agreed with those obtained by direct counts (Thomassen *et al*., 1975).

5B.4.4 *Origin of preneoplastic parent cells*

To determine the origin (progenitor cell) of foreign-body tumorigenesis, histological and electron microscopic studies were carried out with sarcomas induced in CBA or AKR mice by subcutaneous implantation of unplasticized vinyl chloride–vinyl acetate copolymer films and with cultured cells from preneoplastic capsule tissue (Johnson *et al*., 1973a; Buoen *et al*., 1975; Johnson *et al*., 1977, 1980).

In spite of the heterogeneity of sarcoma types obtained, all sarcomas were characterized by (*a*) a pericellular, periodic acid–Schiff-positive, argyrophilic and filamentous substance resembling basal lamina; (*b*) a sparsity of collagen production; and (*c*) prominent cytoplasmic accumulation of 6-nm microfilaments in 60–100% of the cells from each sarcoma. Sarcomas from six mice with leiomyomatous cells, in which extensive concentrations of microfilaments were observed ultrastructurally, also had many acid fuchsin-positive cells when examined with the light microscope. The consistent presence of these morphological characteristics despite the variability of histological sarcoma type suggested that a pluripotential mesenchymal cell type other than the fibroblast is the common progenitor cell (Johnson *et al*., 1973a).

Preneoplastic foreign-body segments (film removed from capsule) or reactive capsule tissue were excised at 1, 1.5, 2, 5 or 10 months after implantation of unplasticized vinyl chloride–vinyl acetate copolymer in CBA mice. Reactive capsule tissue was minced and treated for 30 min with 0.3% collagenase at 37°C. The cells were cultured in 35-mm plastic culture dishes (with an 11 × 22 mm glass coverslip on the floor of each dish) in medium with antibiotics and 20% fetal calf serum. Cell-laden film implants were placed directly in 35-mm culture dishes containing the culture medium. The cultures were passaged routinely (after trypsinization) as soon as monolayers reached confluence (i.e., after approximately four to six days for capsule-derived primary cultures and three to five weeks for film-attached primary cultures). Cells isolated *in vitro* were found to conform to four cell-type categories on the basis of light microscopic morphology, pattern of in-vitro appearance, in-vitro topographical relationships and certain karyotype similarities. Euploid type I (macrophage-like) and type II (fibroblast-like) cells predominated in primary cultures and early passages (passages 1 and 2) of cells derived from reactive capsule tissue. The observation of small numbers of type III cells (unidentified cell type with unknown karyotype characteristics) in passages 1 and 2 of cells derived from reactive capsule

tissue coincided with the deterioration of euploid type II cell populations and preceded the appearance of type IV (endothelial-like) cells. Type IV cells had a pronounced growth advantage over cell types I, II and III, resulting in cultures composed only of type IV cells after three passages. Cultures derived from cells attached to the surfaces of implant segments also conformed to the criteria established for type IV cells. Of the four cell types, type IV cells were determined to have special importance regarding the nature of the progenitor cell in foreign-body tumorigenesis, in that they were aneuploid and eventually produced homologous sarcomas when injected as a suspension into compatible hybrid recipient mice that carried the T6 marker chromosome. [The Working Group noted that details of the experiment on type IV cell injection were not reported.] The growth characteristics of type IV cells were significantly different from those of type II cells, in that the former grew to confluence, forming a monolayer with little or no evidence of piling up or cell overlap. These morphological features and the tendency to form monolayers of uniformly spaced cells in pavement-like or mosaic patterns are consistent with the characteristics reported for cultured endothelial cells *in vitro*. The findings were considered to be consistent with the hypothesis that cells of the local microvasculature are the likely progenitor or parent cells from which foreign-body sarcomas are derived (Johnson *et al*., 1977).

The submicroscopic features of the aneuploid type IV cells included: (1) numerous microfilaments (diameter, 6–9 nm); (2) many plasmalemmal (pinocytotic) vesicles; (3) many surface microvilli, ruffles or blebs; (4) formation of intercellular gap junctions; and (5) relatively extensive smooth-surfaced endoplasmic reticulum and significantly less rough-surfaced endoplasmic reticulum (Johnson *et al*., 1980).

5B.4.5 *Stages in foreign-body tumorigenesis*

On the basis of the results described above, foreign-body tumorigenesis was considered by Brand *et al*. (1975c) and Brand (1976) to be a multistage process. In Stage 1, the foreign body surface was covered with a macrophage monolayer by the 12th day after implantation. Within four to eight weeks, preneoplastic parent cells could be seen in the loose cellular foreign body reactive tissue, but not on the foreign body surface. In Stage 2, a fibrous capsule around the foreign body was formed during the second month after implantation. Preneoplastic cells were then present as clones in the capsule and in the loose connective tissue around it, but still not on the foreign body surface. In Stage 3, preneoplastic clonal cells began to settle on the foreign body surface. The fibrotic consolidation of the capsule was complete between the fourth and sixth months. The macrophage-type cells that still predominated on the foreign body surface appeared ultrastructurally inactive and dormant. However, foreign-body contact was not at that time a necessary condition for preneoplastic cell maturation. In Stage 4, direct foreign-body contact became a requirement for completion of preneoplastic maturation. Homologous preneoplastic cells in the capsule would not give rise to tumours upon transfer of the capsule unless a new foreign body was inserted. In Stage 5, the preneoplastic cells had acquired autonomy. The proliferating cells detached from

the foreign body surface and invaded the capsule tissue. Capsule transfer at this stage could induce tumours in recipient animals. [The Working Group noted that because of the inadequacy of reporting, it is not possible to determine to what extent these conclusions are based on evidence or are conjectural. Their validity cannot always be determined.]

5B.4.6 *Other data on the role of capsule and implant on tumour promotion/progression*

(*a*) *Different roles of an implant during early and late stages of carcinogenesis*

As described in Section 4B.22, the presence of an implant of continuous surface in a tissue capsule is essential for induction of malignant tumours at the site of implantation. However, the data in Table 58 show that the implanted foreign body plays different roles during the early and later stages of carcinogenesis. Non-perforated poly(vinyl chloride) (PVC) film (A) induced local tumours at a significantly higher rate than perforated PVC film having 50–60 holes (0.3 mm wide) per cm^2 (D) or Millipore filter with 0.45-μm pores (E); a significant decrease in local tumour development was seen

Table 58. Replacement of non-perforated poly(vinyl chloride) film by perforated poly(vinyl chloride) film or by Millipore filters

Implant		No. of sarcomas/ no. of mice alive 12 months after implantation	Tumour incidence (%)[a]	Minimal latent period (months after first implantation)
A	Non-perforated film (no replacement)	17/30	56.6	11.5
B	Non-perforated film (removed after 3.5 months)	0/20	0	–
C	Non-perforated film (removed after 6.5 months)	1/29	3.6	10.0
D	Perforated film (no replacement)	3/29	10.3	13.0
E	Millipore filter (no replacement)	1/34	2.9	6.0
F	Non-perforated film → Millipore filter (replaced at 3.5 months)	6/28	21.4	11.0
G	Non-perforated film → perforated film (replaced at 1.5 months)	5/35	14.3	13.5
H	Non-perforated film → perforated film (replaced at 3.5 months)	10/27	37.0	14.0

[a] D and E, significantly less than A ($p \leq 0.0005$); F, significantly greater than E ($p = 0.007$; H, significantly greater than D ($p = 0.011$); B and C, significantly less than A ($p < 0.005$)
Adapted from Moizhess & Vasiliev (1989)

when the non-perforated PVC film (A) was removed after 6.5 months from the capsule formed (B and C). However, when the non-perforated PVC film was replaced with a perforated PVC film (H) or Millipore filter (F) after 3.5 months, the local tumour incidence was significantly higher than that found with perforated PVC film (D) or Millipore filter (E) implanted from the beginning (Moizhess & Vasiliev, 1989).

These data suggest that preneoplastic clones formed in the micro-environment of non-perforated film can continue their neoplastic evolution in the micro-environment of weakly carcinogenic foreign bodies (perforated film, Millipore filter). To test this suggestion, the following experiments were performed. In Experiment 1, non-perforated film implanted into CBA mice (donors) was surgically removed from the capsule and minced into 12–15 fragments. These fragments with attached cells were implanted into F_1(CBA × C57BL) mice (recipients). Five out of 34 recipient mice developed sarcomas at the site of implantation. All these sarcomas were transplantable to CBA and F_1 mice but not to C57BL mice. In the control group, 'intact (fresh)' minced film alone was implanted into F_1 mice and none of 26 mice surviving after 12 months developed sarcomas. In Experiment 2, the capsules that formed around non-perforated film were removed four months after implantation. These capsules were minced into small fragments, mixed with fragments of Millipore filters (fresh) and transplanted subcutaneously into F_1 mice. Five out of 12 recipients developed tumours and all these tumours were of donor origin. Thus, 'preneoplastic' cells present in the capsule around non-perforated film were able to develop into sarcomas when placed into the environment around fragmented Millipore filter. In Experiment 3, the non-perforated film with surrounding capsule was excised after four months in CBA mice and transplanted into F_1 mice. Fourteen days later, the film was surgically removed from the capsule. After another four months, Millipore filters were implanted at the same site. Three tumours developed in 28 mice of this group and they were of CBA origin. No tumours were observed in the control group, in which Millipore filters were not implanted after removal of the film. Thus, film fragments alone were not carcinogenic, but they promoted the development of tumours from the donor cells attached to the film surface before fragmentation (Moizhess & Vasiliev, 1989).

(*b*) *Promotion by an implant of subcutaneous carcinogenesis initiated by irradiation or a chemical carcinogen*

The results presented in Table 59 show that total-body γ-irradiation alone, as well as implantation of perforated film alone, induced a low frequency of subcutaneous sarcomas. Implantation of perforated film 2.5 months after total irradiation induced sarcomas at a considerably higher incidence. This incidence decreased to that of control groups when the film was implanted 1.5 months after irradiation and removed four months after implantation. Thus, the continued presence of the implanted film in the subcutaneous tissue of irradiated mice was needed for efficient tumour formation. Minimal latent periods of tumour formation were shorter in the group of mice irradiated before implantation than in the control group (Moizhess & Vasiliev, 1989).

Table 59. Induction of sarcomas by subcutaneous implantation of perforated poly(vinyl chloride) film after total-body γ-irradiation

Experiment	Experimental conditions	No. of sarcomas/ no. of mice alive after 12 months (%)	Minimal latent period (months)
1A	Perforated film alone	2/33 (6.0)	22
1B	γ-Irradiation (8.2 Gy + bone marrow) + perforated film implanted 2.5 months later	10/34 (29.5)	12
2A	Perforated film alone	8/34 (23.5)	13
2B	γ-Irradiation (7.5 Gy) alone	1/21 (4.2)	16
2C	γ-Irradiation (7.5 Gy) + perforated film implanted 1.5 months later	15/29 (51.7)	8.5
2D	γ-Irradiation (7.5 Gy) + perforated film implanted 1.5 months later and removed after four months	1/24 (4.7)	14

1A, significantly less than 1B ($p < 0.0001$); 2A, significantly less than 2 C ($p < 0.0005$); 2B, significantly less than 2C ($p < 0.00005$); 2D, significantly less than 2 C ($p < 0.00005$)
Adapted from Moizhess & Vasiliev (1989)

In other experiments, a single intraperitoneal injection of *N*-ethyl-*N*-nitrosourea (ENU) was given to CBA mice; 2.5 months later, two foreign bodies were implanted into each mouse: minced PVC film was implanted into the subcutaneous space on the left flank and perforated film on the right flank. Because many CBA mice developed multiple lung adenomas at 9–10 months and died in a preliminary test, in order to increase the observation period, the implanted foreign bodies with surrounding tissues from ENU-treated and from control CBA mice were transplanted into intact two- to three-month-old F_1 mice (CBA × C57BL/6) 9.5 months after the implantation. The incidence of tumours was much higher around the implants from ENU-treated animals than around the implants from non-treated animals, as shown in Table 60. The tumours arising in new hosts were of donor origin (Moizhess & Vasiliev, 1989).

5B.4.7 *Effect of different implant materials on inhibition of gap-junctional intercellular communication as an index of tumour promotion*

Many tumour-promoting chemicals inhibit cell–cell communication mediated by gap junctions (Swierenga & Yamasaki, 1992; Budunova & Williams, 1994). The inhibition of gap-junctional intercellular communication (GJIC) by tumour promoters is thought to play an important role in carcinogenesis (Holder *et al*., 1993; Mesnil & Yamasaki, 1993).

Table 60. Induction of sarcomas by 'non-carcinogenic' film after injection of *N*-ethyl-*N*-nitrosourea (ENU)

ENU	Film	No. of sarcomas/ no. of implants at 12 months	Tumour incidence (%)	Minimal latent period (months)
+	–	0/28	–	–
–	Perforated	5/30	16.6	23
–	Minced	1/27	3.7	19
+	Perforated	12/30	40	10.5
+	Minced	10/31	32.2	10.5

Adapted from Moizhess & Vasiliev (1989)

A short-term GJIC assay, the V79 metabolic cooperation test (Trosko *et al.*, 1982) was used to assess the putative tumour-promoting activity of various non-metallic materials. In these experiments, culture medium extracts from the materials were tested. The test samples were pure poly(ether urethane) (PEU), pure silicone (I-Silicone) and their 1 : 1 blend films (PEU/I-Silicone), the tumorigenic potential of which had previously been evaluated *in vivo* (Nakamura *et al.*, 1992; see Section 4B.2.1). One gram of these materials was cut into small pieces and extracted with 10 mL of the culture medium for 24 h at 37°C, and the extracts were diluted serially with the medium. Undiluted extracts from I-Silicone, PEU/I-Silicone or PEU showed a clear inhibitory effect on GJIC, but their activity varied: the lowest effective dilutions of PEU, PEU/I-Silicone and I-Silicone were 12.5, 25 and 50%, respectively (Tsuchiya *et al.*, 1995a). These in-vitro activities correlated well with the in-vivo long-term (two-year) tissue responses including tumour development following implantation of these materials in rats (Nakamura *et al.*, 1992).

The parameters to describe the histological response, including tumour development and cell proliferation with or without preneoplastic change following implantation in rats of three PEUs of the same chemical composition but of different molecular weight (Nakamura *et al.*, 1992) did not correlate with the lowest effective concentrations of methanol extracts of these materials in the metabolic cooperation inhibition test. However, they were highly correlated ($r = 0.99$) with the inhibitory activity of the materials themselves when they were used to coat culture dishes at 2 mg per 22-mm diameter dish. In the same studies, various amounts of S-silicone (an uncured vinylmethylpolysiloxane mixed with 25% silica) were coated on the surface of glass dishes for the metabolic cooperation assay. The results with S-silicone were equivocal, indicating that the inhibitory potential of the S-silicone-coated dish was weaker than those of the PEU-coated dishes (Tsuchiya & Nakamura, 1995).

Components of the PEU were tested to identify which chemical structure(s) inhibited GJIC. A carbamate, 4,4′-di(ethoxycarboamide)diphenylmethane (MDU), which

is considered to be a model of the hard segment of PEUs, was not active in this assay while 1,4-butanediol (a chain extender of polyurethane) inhibited metabolic cooperation only at very high concentrations (> 600 μg/mL). Four different kinds of poly(tetramethylene oxide) (PTMO) (a component monomer of the soft segment) were active at lower concentrations than 1,4-butanediol. At a concentration of 10 μg/mL, the PTMO of the lowest molecular weight tested had the highest activity (Tsuchiya & Nakamura, 1995; Tsuchiya *et al*., 1995a).

Other types of polyurethane (PUs), such as fluoropolyether glycol (FPEG)-PU, polybutadiene (PBD)-PU, and hydrogenated PBD (HPBD)-PU, were tested by the coated dish method. Surprisingly, these materials were either inactive or were very weak inhibitors of GJIC. The lowest effective amount of FPEG-PU was 8 mg per 22-mm diameter dish, which was 10-fold greater than that of the most active of the PEUs tested above. FPEG-PU possesses a side-chain substituted with fluoride, and this side-chain might hinder oxidation and reduce biodegradability (Takahara *et al*., 1991). PBD-PU and HPBD-PU, which contain no polyether moiety, showed no inhibitory activity in the assay. From these results, it was speculated that the polyether moiety such as PTMO is a key structural feature in the inhibition of GJIC by PEUs (Tsuchiya *et al*., 1995b). No in-vivo tumorigenicity data for these materials are available.

The GJIC of V79 cells was inhibited on the surface of polyethylene film, but this inhibitory activity was markedly decreased when the surface of the polyethylene film was immobilized with collagen. This decrease was dependent on the amount of immobilized collagen on the polyethylene film (Nakaoka *et al*., 1997). On the other hand, collagen immobilization greatly reduced the tumour incidence induced by polyethylene sponges in a one-year subcutaneous implantation study (Kinoshita *et al*., 1993). Again, a good in-vivo/in-vitro correlation was obtained.

In contrast, the GJIC inhibitory activity of polyethylene film in the metabolic cooperation assay was only slightly reduced when the polyethylene was immobilized either with bovine serum albumin or with Arg-Gly-Asp-Ser (RGDS) peptide, a known sequence of the cell attachment domain in extracellular matrix proteins. This indicates that cell attachment to the polymer via the RGDS peptide was not a dominant factor for GJIC. The reduction of the inhibitory activity of GJIC by collagen immobilization seems to be due not only to improvement of the cell adherence onto polyethylene film via an RGDS sequence but also to some other interaction between collagen and cells which is essential for normal assembly and function of connexins. Generally, the surface modification of polyethylene by means of collagen immobilization may provide an environment suitable for maintaining normal cell function (Nakaoka *et al*., 1997).

It has been reported that collagen immobilization on silicone elastomer before subcutaneous implantation into rats led to strong adhesion with the surrounding tissue and prevented down-growth of epidermal tissue and bacterial infection (Okada & Ikada, 1995).

On the basis of these results, a hypothesis concerning the tumorigenic potential of polymeric materials has been presented. The genotoxic activities of these materials are

probably weak but their tumour-promoting activities may be significant and may differ between materials. Greater promoting activity will affect tumorigenic potential by shortening the latency period and increasing tumour incidence, and may be mediated by differences in the GJIC-inhibiting potential of the surfaces of the materials (Tsuchiya & Nakamura, 1995; Tsuchiya, 1998; Tsuchiya *et al*., 1998).

5B.4.8 *What initiates the formation of preneoplastic parent cells?*

It has been hypothesized that, in mice, a primary 'transforming' event takes place before the fourth to eighth week of the foreign-body reaction, and that such transformation may occur at sites distant from the foreign body, in a single or a few mesenchymal pluripotential stem cells of the microvasculature (Brand, 1976) [The Working Group noted that the existence of such cells is no longer believed]. Several possibilities for the nature of this initiating event were proposed: (*a*) a genome change caused either by mutation or activation of indigenous viral genes, (*b*) chromosome defects or imbalance and (*c*) aberrant epigenic differentiation (Brand, 1976).

Viruses or viral genomes have been sought in cells of foreign body-induced tumours, but no convincing evidence of a causal relationship has been found.

The possibility that mutagenic chemicals leach out of the materials and initiate parent cells by a process of chemical carcinogenesis seems unlikely in most cases, because the conventional mutagenicity assays (bacterial gene mutation and mammalian cell chromosomal aberration assays) gave negative results with PEUs, PVC, poly(2-hydroxyethyl methacrylate) and silicone, when the leachables obtained by extraction with organic solvents followed by evaporation were tested (Nakamura *et al*., 1992). This conclusion is supported by the finding that subcutaneous implantation of powdered polymers did not induce tumours, although leaching of chemicals from pulverized polymers is accelerated.

Possible tumour-initiating activities of polyurethane materials were tested using the two-stage BALB/c 3T3 cell transformation assay. Firstly, methanol extracts of PEU were tested in the presence or absence of TPA, a potent tumour promoter. The extract alone did not increase the number of transformed foci, but did in the presence of TPA. These results indicate that leachable substances from PEU can act as a weak tumour initiator, while transforming activity was found only in the presence of TPA. In the same series of experiments, chemical models of component units of PEUs were tested to identify a chemical moiety that might induce the initiation of cell transformation. 1,4-Butanediol induced no significant increase in transformed cell foci up to a concentration of 20 mg/mL. PTMO of average molecular weight 1000 also showed no transforming activity, whereas MDU induced transformation at a concentration of 0.2 μg/mL. Therefore, it is possible that substances containing a phenylcarbamate structure act as tumour initiators. In order to clarify the effects of direct interaction of cells with the coated materials on cell transformation, one half of the surface of a set of test dishes was coated with PEUs. The number of foci on the area coated with PEU increased significantly in the presence of TPA, whereas no increase

was found on the non-coated area. These results imply that initiation may be caused not only by the leachable substances but also by biodegradable substances present on the surface of PEUs. Comparison of the potencies of tumour-initiating and -promoting activity of the PEUs in the various assays indicated that the promoting activities of the PEUs are stronger than the initiating activities.

From the results described, it was speculated that the mechanism of tumorigenesis induced by PEUs involves (1) initiation caused by the hard segment, with a chemical structure like that of MDU (this chemical moiety is present in the leachable and biodegradable oligomers) and (2) promotion mainly through the action of a polyether soft segment moiety such as PTMO in inhibiting GJIC. This soft segment moiety is derived from the leachable oligomers and degradation through a direct cell/material interaction (Tsuchiya *et al*., 1996).

Various tumour promoters have been shown to inhibit GJIC by phosphorylation of connexin proteins. The effects of PEUs were investigated on connexin 43 (Cx43), a major gap-junction protein of both V79 and BALB/c 3T3 clone A31-1-1 cells. Cx43 in the A31-1-1 cells after transformation by PEUs was phosphorylated at tyrosine, serine and threonine residues and GJIC was inhibited, according to the dye-transfer assay. Both hard and soft segment models induced phosphorylation of Cx43 and also inhibited GJIC, which is considered to play an important role in tumour promotion (Tsuchiya *et al*., 1998). Thus, a post-translational modification or a defect in gene expression, but not a mutation in the coding sequence of the *Cx43* gene is probably involved in the PEU-induced inhibition of GJIC during tumorigenesis *in vitro* (Tsuchiya *et al*., 1999). However, since it is not clear how GJIC regulates cell growth, further studies on the molecular mechanism of the inhibitory action on GJIC by interaction of cells with biomaterials are needed.

5B.4.9 *Possible genotoxic mechanisms underlying solid-state carcinogenesis*

To investigate the relationship between inflammatory processes and foreign-body carcinogenesis, groups of 20 male Fischer 344 rats, four months old, were given implants of discs (15 mm diameter × 0.2 mm) of silicone elastomer prepared with dichlorobenzoyl peroxide catalyst, as used in breast implants (Dow Corning), or cellulose acetate filters (Millipore) with pore sizes of < 0.02 μm ('impermeable', positive control) or > 0.65 μm ('porous', negative control) into a dorsal interscapular subcutaneous pocket (James *et al*., 1997). Groups of 10 rats were killed one week or two months after implantation. Each implant and tissue capsule was divided for histopathological examination, for immunohistochemical determination of leukocyte antigen expression and cell proliferation and for in-situ 3′-OH-end-labelling to measure DNA strand breaks in DNA-damaged viable cells and in apoptotic cells. Inflammatory response and capsular thickness were assessed blind, using qualitative scales. Similar acute inflammatory responses, seen in both the silicone and positive-control groups, comprised a discontinuous layer of macrophages adjacent to the implants and a loosely organized capsule of collagen fibres interspersed with nume-

rous spindle cells and neovascularization after one week. The response in the negative-control group comprised a continuous layer of macrophages, mainly multinucleated syncytial giant cells, closely adherent to the implants, with fibrillar processes penetrating the pores and only a weak fibrotic response. By two months, dense capsules of linearly aligned collagen fibres with minimal vascularization, numerous spindle cells and minimal lymphocytes or macrophages were evident in the silicone and positive-control groups. In the negative-control group, the macrophage layer and degree of fibrosis were similar to those observed before. A significant ($p < 0.001$) difference in capsular thickness was observed between the negative-control group (0.08 mm) and the other groups (0.27 and 0.21 mm for the silicone and positive-control groups, respectively).

There were no clear differences in the expression of leukocyte antigen epitopes at one week; CD11b/c (a marker for phagocytic cells exposed to cytokines) was strongly expressed in the macrophage layer in all groups. CD4 and CD8 epitopes (expressed by T helper/inducer and T cytotoxic/suppressor cells, respectively) were scattered throughout the capsule. After two months, expression of CD11b/c, CD4 and CD8 was reduced in the silicone and positive-control groups but remained broadly similar to that seen in week 1 in the porous-filter group, indicating that the high level of these inflammatory markers was maintained with the non-carcinogenic material only.

Serial sections of formalin-fixed tissue were labelled and stained for 3′-OH of DNA strands by in-situ end-labelling (see Table 61). The percentage incidence of stained spindle cells was determined in fields adjacent to the implant and in the multinuclear giant-cell layer. At one week, DNA fragmentation was common in viable cells in capsules surrounding the silicone and positive-control materials (52 and 53.3%, respectively) but rare in the negative-control group (0.2%; $p < 0.01$ relative to other groups). Labelled cells were interspersed throughout the capsule but did not

Table 61. Percentage of viable and apoptotic cells detected by in-situ end-labelling of DNA in connective tissue adjacent to implants

		Silicone	Positive control	Negative control
1 week	% viable cells with DNA strand breaks	52.0 ± 8.4	53.3 ± 3.3	0.2 ± 0.1[a]
	% apoptotic bodies	4.7 ± 1.0	12.1 ± 1.4	2.1 ± 0.6[a]
2 months	% viable cells with DNA strand breaks	50.8 ± 5.9	49.8 ± 6.9	0.1 ± 0.02[a]
	% apoptotic bodies	11.0 ± 1.2	4.9 ± 0.2	0.9 ± 0.2[a]

[a] $p < 0.01$ relative to silicone and positive control
Adapted from James *et al*. (1997)

coincide with markers for leukocyte antigen epitopes, such as CD11b/c. A similar pattern was evident at two months. These DNA-damaged cells were assumed to originate from connective tissue.

A similar method was used to quantify cell proliferation through immunohistochemical analysis of proliferating cell nuclear antigen (PCNA). After one week, cell proliferation was higher in spindle cells and macrophages in capsules surrounding the silicone and positive-control materials (36.1 and 33.7%, respectively, for spindle cells) in comparison with the negative-control group (13.9%). By two months, the proportion of proliferating cells had decreased to about 6% (1.6% in negative controls), and these were predominantly spindle cells within the well-defined capsule.

The authors concluded that cell proliferation, apoptosis and DNA strand breaks were significantly increased in tissue adjacent to carcinogenic implants. Conversely, in the presence of non-carcinogenic implants, DNA strand breaks were negligible and associated with reduced levels of proliferation and apoptosis. [The Working Group noted that the presence of DNA strand breaks was not confirmed by a separate assay. The immunohistochemical detection system used (Apotag) is usually interpreted as indicating DNA fragmentation related to apoptosis, not to genotoxicity, as suggested by the authors].

The authors of these studies emphasized the similarity of the pathology observed in the silicone and positive-control group, in which the acute inflammatory response was attenuated and replaced by fibrosis, and the different cellular response produced by the porous material, in which the inflammatory response was chronically maintained and associated with minimal fibrosis. The frustrated phagocytic response, typified by syncytial giant-cell aggregation around the porous material, was in contrast to the fibrotic response towards impenetrable materials, directed at isolation of the foreign body. The authors hypothesized that this could be the result of specific cytokine release. It was further postulated that the loss of vasculature associated with capsule formation would lead to an environment known to promote carcinogenicity. The authors concluded that the results suggest that the micron-scale surface morphology of the implant determines the nature of the subsequent cellular response, which may predispose to tumour development (James *et al*., 1997).

Further work on tumour development, reported in an abstract, investigated the incidence of mutations in the *p53* gene in human tissue. Experimental groups were apparently similar to those reported above, with implants of silicone elastomer and non-porous cellulose acetate, although no further information on this was given. Within 12 months of implantation, hyperplastic inflammatory foci were observed within the fibrous tissue adjacent to the carcinogenic implants. These foci stained positive for both mutant p53 protein and DNA strand breaks, as tested by *in situ* end-labelling. Fibrosarcomas developed in all rats in these groups within two years of implantation. No tumours developed in negative controls. Polymerase chain reaction (PCR) amplification and single-strand conformation polymorphism (SSCP) analysis of DNA from tumour tissue samples indicated that 40% of tumours (6/15) exhibited

abnormal band migration patterns consistent with *p53* gene mutation. Reamplification and direct sequencing of an abnormal SSCP band revealed a double mutation in exon 6 of the gene (C→T, G→T). Further analysis revealed a hot-spot mutation in 50% of the tumours with *p53* mutations, comprising GCT→CCC (Ala→Pro), at codon 201 of exon 6. The authors concluded that these results suggest, for the first time, that indirect genotoxic mechanisms resulting in *p53* mutations are involved in foreign-body carcinogenesis (Pogribna *et al*., 1997). [The Working Group noted that *p53* mutations may arise during the later stages of tumour development.]

5C. OTHER FOREIGN BODIES

5C.1 Degradation in biological systems

No data were available to the Working Group.

5C.2 Distribution and excretion

The potential for components of implanted bullets and shell fragments to be mobilized (solubilized or degraded by phagocytosis) and distributed to distant parts of the body is relevant to the systemic carcinogenicity of such foreign bodies. Of the possible metals in these objects, lead and depleted uranium are those of greatest concern.

5C.2.1 *Lead*

(*a*) *Humans*

That lead is distributed from the site of retained bullets to other areas of the body has been clearly demonstrated from the number of case reports of lead toxicity in individuals with retained bullets.

Machle (1940) reported in detail on two cases in which clinical diagnoses of lead poisoning were correlated with retained bullets. He further reviewed 40 other cases that had been described in the literature from 1867 to 1938 and was impressed with the paucity of cases compared with the frequency of gunshot wounds and of bullets that had been permitted to remain in the body. Bird and buck shot accounted for a fairly high proportion of the cases, even though it is likely that many more persons had implanted artillery shrapnel and rifle bullets from the First World War. The interval between lodgment of the bullets (including buckshot) and initial lead poisoning symptoms varied considerably (from 12 days to 48 years), although among the 13 cases in which the symptoms developed in less than one year, only two would be considered cases of lead poisoning at the present time. The location of lodgment seemed important, as more than half of the bullet-related lead poisoning cases consisted of bullets retained within bones or joints.

Since Machle's review, a number of individual case reports of bullet-related lead poisoning have been published. In 1982, Linden *et al*. (1982) presented three additional bullet-lead poisoning cases plus a review of 13 other cases. Additional sporadic

reports of bullet retention and resultant lead poisoning have been reported since that time.

In a more recent review and critical analysis of lead poisoning associated with retained bullets, Magos (1994) analysed the data presented by Machle (1940), Linden *et al*. (1982) and other published reports. On the basis of this extensive review, the author concluded that while it is likely that only a fraction of persons with implanted lead projectiles actually develop lead poisoning, it is even more likely that only a fraction of those with bullet-related lead poisoning were actually diagnosed with the condition. The number of mild lead poisoning cases is probably quite high but many were missed by the examining clinicians due to (1) non-specificity of signs and symptoms of lead poisoning, (2) general lack of awareness and familiarity as to the toxicity of lead and (3) inappropriate use of laboratory tests of lead indicators (blood, serum, urine analysis). Mobilization of lead from retained bullets and shot may be influenced by several factors, depending on mobilization from either the projectile itself or from the surrounding tissues and other secondary storage sites. The factors that can be considered most important are indicated in Table 62. On the basis of the cases reviewed, the risk of lead poisoning and the latent period could not be predicted, but it was noted that the number of known clinical cases was small in relation to the actual number of persons carrying lead-containing bullets (Magos, 1994).

(*b*) *Experimental systems*

Discs of lead (enriched with two natural isotopes) were implanted into the knee joints or leg (thigh) muscle of two mongrel dogs. The animals were monitored by mass spectrometry for release of lead in blood over a three-year period. ^{206}Pb served as a marker for the discs implanted into the synovium, while ^{208}Pb served as the marker for lead implanted in the muscle. The knee implant underwent vigorous attack by the synovial fluid and blood lead levels reached a maximum in four to six months, declining thereafter as the remaining fragments became encapsulated. In contrast, there was only minor mobilization of lead from the discs placed in the muscle during the first month and even less thereafter as the discs became encapsulated. Very little physical change was noted in the muscle implants, whereas the joint implants had disintegrated after six months into a number of particles with corroded outlines. The smaller particles subsequently became encapsulated within the joint (Manton & Thal, 1986).

5C.2.2 *Depleted uranium*

(*a*) *Humans*

The distribution of uranium was determined radiochemically in tissues obtained at autopsy of a man who had been employed in the uranium processing industry for 26 years. The deposition of uranium in human tissues followed the order: skeleton > liver > kidney, with concentration ratios of 63:2.8:1. This study indicates that the long-term storage compartment for uranium in the skeleton may be greater than previously estimated (Kathren *et al*., 1989).

Table 62. Factors that may affect the mobilization of lead from retained bullets

Mobilization from the projectile (lead bullet)	
Surface area	Dissolution is faster from multiple pellets than from an equal mass of a single bullet and is faster from fragmented bullets than from non-fragmented ones.
Location	Bullets retained in soft tissues tend to become encapsulated by fibrous tissue which impedes release of lead.
Mechanical effects	Impact of bullet with bone creates abrasive effect on bullet lodged in a joint, which promotes disintegration of the bullet.
Acidity	Low pH of synovial and bursal fluids promotes dissolution, with high lead concentrations in the fluids and surrounding tissues.
Mobilization from surrounding tissues and other secondary storage sites	
Inflammation	Lead taken up by the surrounding tissues may cause synovitis and arthritis, which will promote dispersal of lead to other areas. Cell migration and increased blood flow may also play a role.
Impaired use of limbs	Inactivity due to painful arthritis can promote mobilization of lead from bones.
Hypermetabolic conditions	Alcoholic acidosis, hyperthyroidism and fever may promote lead mobilization and increase sensitivity to lead.

Modified from Magos (1994)

(*b*) *Experimental systems*

The distribution of implanted depleted uranium was studied with Sprague-Dawley rats using three dose levels (low, medium, high: 4, 10, 20 pellets). The implants consisted of 99.25% depleted uranium and 0.75% titanium with the uranium isotopes amounting to 99.75% ^{238}U and 0.2% ^{235}U and trace levels of ^{234}U. [The Working Group noted that the authors did not consider the radioactivity of the residual ^{235}U and ^{234}U isotope as a major concern.] The pellets were implanted into the gastrocnemius muscle of male Sprague-Dawley rats and tissue samples were analysed at day 1 and at 1, 6, 12 and 18 months. Within one day, uranium had appeared in the kidney and bone. By six months, the uranium level had reached a plateau in the kidney but continued to rise in bone throughout the 18-month period in the high-dose group. The urine concentration of uranium reached a maximum at 12 months and had declined by 18 months. A dose- and time-related increase in uranium levels was found in many tissues. The greatest concentrations were found in the kidney and bone, the primary reservoirs for uranium redistributed from intramuscularly embedded depleted uranium

fragments. Many tissues other than muscle had significant concentrations of uranium, including the brain, liver, spleen, lung, lymph nodes and testes (Pellmar *et al*., 1999).

The effects of implantation of depleted uranium pellets were studied in Sprague-Dawley rats. Groups of animals received 20 depleted uranium pellets (high dose), 10 depleted uranium pellets and 10 tantalum (inert control) pellets (medium dose), or four depleted uranium and 16 tantalum pellets (low dose). The control group received 20 tantalum pellets. At 6, 12 and 18 months after implantation, the concentrations of uranium in urine were significantly increased in all dose groups, peak concentrations being observed at 12 months (Miller *et al*., 1998a). In the same study, mutagenicity in urine was investigated (see Section 5C.4.2).

5C.3 Tissue responses and other expressions of toxicity

5C.3.1 *Lead*

The toxicity of systemically distributed lead is well known and has been reviewed by IARC (1980) and elsewhere. The toxic effects in humans involve several different organ systems with subtle clinical symptoms in most cases. In adults, the main organs affected are the neurological system, the haem-synthesizing system and the kidneys. With excess occupational exposure or accidental exposures to lead, the most evident effects have been peripheral neuropathy and chronic nephropathy. The most sensitive effects in adults may be hypertension and anaemia. Less commonly, lead-induced toxicity may affect the gastrointestinal and reproductive systems (sterility and neonatal deaths).

5C.3.2 *Depleted uranium*

In humans, the kidney and bone are the primary target organs of internal exposure to uranium, regardless of the route of exposure. Most of the absorbed uranium is cleared from the blood stream and excreted in the urine within 24 h. The uranium that is not excreted is reabsorbed by the proximal tubules of the kidney, where it causes its primary toxic effects (Kathren *et al*., 1989; Pellmar *et al*., 1999). Chronically exposed uranium mill workers showed mild dysfunction of the kidney and increased urinary excretion of beta-2-microglobulin (Thun *et al*., 1985). In one case study, neurological effects were seen (Goasguen *et al*., 1982). These data indicate that embedded fragments of depleted uranium may lead to neural damage, which may affect both cognitive and motor functions.

Preliminary results have been published of toxicity studies in rats with implanted depleted uranium. Depleted uranium pellets (1×2 mm) consisting of 99.25% depleted uranium and 0.75% titanium by weight were implanted at three dose levels (4, 10 and 20 pellets) into the gastrocnemius muscle of male Sprague-Dawley rats. Clinical and laboratory analyses are being performed to detect kidney, behavioural and neural toxicity. Six months after implantation, decreased weight gain, a dose-related increase in levels of depleted uranium in the kidneys, bone and urine, and a decrease in neuronal excitation in the hippocampus were reported. However, while uranium was found in the

brain, no behavioural toxicity was observed at six months after implantation. The authors indicated that the kidney toxicity was less than would be expected on the basis of the uranium levels in the kidneys (Pellmar *et al*., 1998).

A preliminary report was given of an ongoing study to assess the potential carcinogenicity of long-term exposure of rats to implants of depleted uranium (DU), such as shrapnel in wounds. Groups of 50 male Wistar rats were given implants into the thigh muscle of 5.0 × 5.0 × 1.5-mm or 2.5 × 2.5 × 1.5-mm DU squares composed of 99.25% uranium and 0.75% titanium. Other groups were given implants of 2.0 × 1.0-mm diameter DU pellets, tantalum squares (negative controls) and thorotrast (thorium dioxide) injections (positive controls). After 15 months of the planned 24-month exposure period, a marked local tissue reaction (including fibrous capsule formation) had developed around the DU and tantalum implants, the capsules being much thicker around the uranium implants. There was also a decrease in weight of the group that received the largest mass of DU, although survival was not affected in any of the groups. Carcinogenic response is not yet known (Hahn *et al*., 1999).

5C.4 Genetic and related effects

5C.4.1 *Lead*

The genetic and related effects of lead have been reviewed (IARC, 1987c).

5C.4.2 *Depleted uranium*

(*a*) *Humans*

No data were available to the Working Group.

(*b*) *Experimental systems*

Mutagenicity induced by depleted uranium implants has been demonstrated in experiments with male Sprague-Dawley rats. Urine and serum of these animals were evaluated for mutagenic potential using the Ames *Salmonella* mutation assay. The implants consisted of pellets implanted into the gastrocnemius muscle at three dose levels. Tantalum was used as a negative control. Urine and blood were collected at 0, 6, 12 and 18 months for the mutagenicity assay. While no mutagenicity was observed with the sera, a substantial dose- and time-dependent increase in mutagenicity was seen with urine samples. Positive results were obtained with *S. typhimurium* strain TA98 and the Ames II™ mixed strains (TA7001-7006). A significant elevation in mutagenic potential was observed in TA98 strain and Ames II™ tests with the Amberlite XAD-4 and XAD-8 column fractions of urine, which was dependent on both the length of time since implantation and the number of uranium pellets implanted. Urine from animals that had tantalum implants showed no increase in mutagenicity. A strongly positive correlation was observed between urinary mutagenicity and urinary uranium levels at 6, 12 or 18 months after pellet implantation (Miller *et al*., 1998a).

A doubling of sister chromatid exchanges was found in human osteoblast-like cells treated with 10 μM of depleted uranium-uranyl chloride for 24 h. This was a

greater response than that found with the positive control, nickel sulfate (Miller *et al*., 1998b).

In the same series of experiments, in-vivo transformation of human osteoblast-like cells with depleted uranium was demonstrated. Human osteosarcoma cells (HOS TE85, clone F-5) were treated with depleted uranium-uranyl chloride (10 μM) for 24 h, at which time the cells were rinsed, trypsinized and seeded onto tissue culture dishes. The dishes were incubated for five weeks and examined for the appearance of transformed foci. Morphologically, the uranium-exposed cells developed into diffused type II foci. A 10-fold increase in transformation frequency was observed in the treated compared to the non-treated cells. The transformation response was stronger with depleted uranium than with the positive controls (nickel sulfate or lead acetate). The transformed cells showed increased expression of the K-*ras* oncogene, and suppression of the phosphorylation of the Rb protein. Transformation was confirmed by injection of 1×10^6 or 5×10^6 of uranyl chloride-treated cells subcutaneously into four- to five-week-old female athymic mice. Tumours developed within four weeks. The histological appearance resembled a carcinoma characterized by an undifferentiated, sheet-like growth (Miller *et al*., 1998b).