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# Chromium accumulation and ultrastructural changes in the mouse liver caused by stainless steel corrosion products

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Stainless steel (SS) corrosion products were obtained by electrochemical dissolution of SS type AISI 316L. Mice were injected subcutaneously with 0.5 ml of SS solution (containing 283 μg Fe, 69.3 μg Cr and 57 μg Ni) each 72 h, for 10 days or 14 days. After the treatment time, livers were removed and were analysed for: (a) liver wet weight; (b) contents in Fe, Cr and Ni; (c) histological and ultrastructural alterations. Results showed that the percentage of liver weight per animal body weight was significantly higher ($p < 0.05$) in SS-injected animals than in the control animals. The atomic absorption spectrometry analysis of dry livers showed that chromium, but not iron or nickel, had a significant increase ($p < 0.05$) in SS-treated mice compared to the control animals. No histopathological differences between 10 and 14 days of SS-injection could be detected, however, massive hepatic degeneration was observed in both groups when compared to the control. These histological changes in SS-treated mice were confirmed at the ultrastructural level, as hepatocytes exhibited an augmentation of vacuoles in their cytoplasm. These actual liver morphological alterations suggest that the hepatocyte function may be hampered, which constitutes a matter of some concern since liver is a blood filtering organ.

## 1. Introduction

Stainless steel (SS) is a metallic alloy widely used in orthopaedic surgery. Quantitative analysis of metals in tissues attached to SS implants have shown accumulation of the main alloying elements iron, chromium and nickel [1]. The release of metal ions from metallic alloys, their transport in the body, and excretion have been a matter of intense research in patients with knee or hip prothesis [2–9] and in animal models [10–16]. Furthermore, injection of stainless steel corrosion products in mice have shown to cause morphological alterations in male reproductive organs [17]. More recently, it has been described that such corrosion products cause alteration of spleen cellular populations, in particular, accumulation of multinucleated giant cells and depletion of lymphocytes [18]. In the present work the toxicity of stainless steel corrosion products were evaluated not only by the accumulation of iron, chromium and nickel in mice liver but also by the histological and ultrastructural changes of the hepatic parenchyma.

## 2. Materials and methods

### 2.1. Animal treatments

Male Charles River mice (2 months old) purchased from "Instituto Gulbenkian de Ciência" (Oeiras, Portugal) were used in this study. Animals were housed at 22 ± 2 °C under normrm laboratory lighting conditions and maintained on a commercial pelleted diet and water *ad libitum*. They were left one week for acclimatization.

A suspension of metallic elements was obtained by electrochemical dissolution of stainless steel (SS) type AISI 316L into Hanks's Balanced Salt Solution (HBSS, Sigma) using a current density of 0.5 mA/cm$^2$. The final contents of metal ions in the SS solution were determined by atomic absorption spectrometry (AAS), as follows: : 6.8 μg/ml Fe, 138.6 μg/ml Cr, and 114.7 μg/ml Ni.

Animals were randomly divided into four groups. Mice of group I ($n = 12$) received subcutaneous injection of 0.5 ml of SS solution each 72 h, for 10 days. Mice of group II ($n = 12$) had the same treatment but for 14 days. Mice of groups III ($n = 10$) and IV ($n = 10$) received an equal volume of the vehicle (HBSS) for 10 and 14 days, respectively. As no differences were observed between groups III and IV, data of both groups were pooled and are presented in "Results" as control (or HBSS-injected) animals.

Animals were observed daily for mortality, and general physical condition. On day 10 and 14,

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mice were weighed and sacrificed by cervical dislocation.

## 2.2. Atomic absorption sepctrometry analysis

For determination of metal ions (Fe, Cr, and Ni) in mice livers, the organs of the four animal groups ($n = 7$ per group) were carefully removed, kept in four plastic tubes and frozen at $-20\,^{\circ}C$ immediately. Prior to AAS analysis, several steps were carried out to eliminate the water and organic matter present in livers. First, the frozen livers were allowed to thaw, placed inside Teflon vessels and dried in a microwave oven model CEM MDS 2000, using a four-stage drying programme as described previously [18]. To control the degree of liver dryness, the samples were weighed after each drying stage. Only when the difference between consecutive stages was lower than 0.0001 g, were the samples assumed to be sufficiently dry.

Dry livers were attacacd with 5.0 ml of $HNO_3$ super pure, purchased from Merck and placed inside the microwave oven to promote their digestion, using a four-stage digestion programme established previously [18]. The resulting residues were dissolved in triply distilled water to a final volume of 25.0 ml. These solutions were then used to determine the metal ions levels by AAS. Iron ion analysis was carried out on a flame chamber Model 357 whereas chromium and nickel were analysed on a graphite furnace from a Perkin Elmer Model 4100ZL, using the working programme previously established [18]. The standards were made in $HNO_3$ solution. The minimal detection limit for iron was 0.04 ppm, for chromium 0.5 ppb and for nickel 1.0 ppb.

## 2.3. Histological and ultrastructural studies

For histological studies, small fragments of liver were fixed in Bouin's solution, embedded in paraffin wax, sliced into 4–5-μm-thick sections and stained with haematoxilin and eosin.

For ultrastructural observation, small pieces of hepatic tissue were fixed in 2% glutaraldheyde, dehydrated and embedded in Epon. Thin sections were made in a Reichert Jung ultramicrotome Model Super Nova, using a diamond knife. Selected ultrathin sections were mounted on Formvar-coated copper grids and counterstained with lead acetate and uranyl citrate. Observation and photographs were performed in a 10C EM Zeiss transmission electron microscope at a voltage of 60 kV.

## 2.4. Statistical analysis

Student's $t$-test was applied to determine the statistical significance of the differences observed between means of the two groups: $p$ values of $p < 0.05$ were considered to be significant.

# 3. Results

## 3.1. Liver weight and metals accumulation

The general appearance of the SS-injected or untreated mice groups were not changed throughout this study. In addition, no macroscopic alterations were perceptible in the liver of SS-injected animals, as the normal lobular structure was maintained and no visible macroscopic intralobular inflammatory reaction was seen.

Furthermore, no significant differences ($p > 0.05$) in body weight or in liver wet weight were found between SS-injected animals and the control group (HBSS-injected animals) as presented in Table I. However, the percentage of liver wet weight per animal body weight showed a significant difference ($p < 0.05$) between SS-injected animals (either 10 or 14 days) and the control group (Table I).

The atomic absorption spectrometry analysis of dry livers showed that chromium, but not iron or nickel,

TABLE I Body weight, liver weight and liver weight percentage in control and SS-injected mice

| Injection | $n$ | Body weight (g) | Liver weight (g) | Percentage of liver weight |
|---|---|---|---|---|
| HBSS (control) | 20 | $24.9 \pm 0.6$ | $1.1 \pm 0.1$ | $4.3 \pm 0.4$ |
| SS (10 days) | 12 | $24.8 \pm 0.5$[a] | $1.2 \pm 0.1$[a] | $4.8 \pm 0.4$[b] |
| SS (14 days) | 12 | $25.2 \pm 0.8$[a] | $1.3 \pm 0.2$[a] | $5.2 \pm 0.5$[b] |

[a] No significant differences ($p > 0.05$) between SS-treated and HBSS (vehicle control) groups.
[b] Significant differences ($p < 0.05$) between SS-treated and HBSS (vehicle control) groups.

TABLE II Concentrations of metal ions in dry weight mice livers

| Injection | $n$ | Fe (μg/g) | Cr (μg/g) | Ni (μg/g) |
|---|---|---|---|---|
| HBSS (control) | 14 | $680.5 \pm 86.9$ | $0.542 \pm 0.088$ | $1.186 \pm 0.400$ |
| SS (10 days) | 7 | $571.5 \pm 25.5$[a] | $2.287 \pm 0.739$[b] | $1.029 \pm 0.085$[a] |
| SS (14 days) | 7 | $706.3 \pm 34.0$[a] | $2.134 \pm 0.953$[b] | $0.934 \pm 0.326$[a] |

[a] No significant differences ($p > 0.05$) between SS-treated and HBSS (vehicle control) groups.
[b] Significant differences ($p < 0.05$) between SS-treated and HBSS (vehicle control) groups.

had a significant increase ($p < 0.05$) in SS-treated mice (either 10 or 14 days) compared to the control animals (Table II). Together, these results indicate that the SS treatment of mice caused accumulation of chromium in liver, which was accompanied by an increase in the percentage of liver wet weight.

### 3.2. Histological and ultrastructural alterations

Observation of liver histological sections from SS-injected mice (10 and 14 days) revealed substantial alterations compared to those of HBSS-injected mice (control group). In contrast, no histopathological differences between SS-treated mice could be detected at 10 and 14 days. In fact, degeneration of the hepatic parenchyma was observed in both groups of animals (Fig. 1). However, regular features of the hepatocyte cord structure were maintained in both treated mice groups. Hepatocytes exhibited a very swollen and clear cytoplasm, and nuclei had a hyperchromatic appearance. The portal track (hepatic portal vein, hepatic artery and bile duct) showed no sign of morphological injury.

At the ultrastructural level, hepatocyte nuclei from SS-injected mice evidenced a regular morphology, but smooth endoplasmic reticulum was decreased when compared to the ones in the control group (Fig. 2). Similarly, the hepatocyte cell surface was coated with numerous microvilli forming some canalicules among them (Fig. 3a). Cytoplasm of these cells evidenced various vacuoles, as described above for histological sections (Fig. 3a). No appreciable morphological changes among bile ductule epithelial cells were noted (Fig. 3b).

## 4. Discussion

These experimental studies indicate that liver is significantly affected d subcutaneous administration of stainless steel corrosion products into adult mice. The increase in the percentage of liver wet weight per animal body weight accompanied the preferential accumulation of chromium rather than iron and nickel. Chromium storage may have contributed to the liver

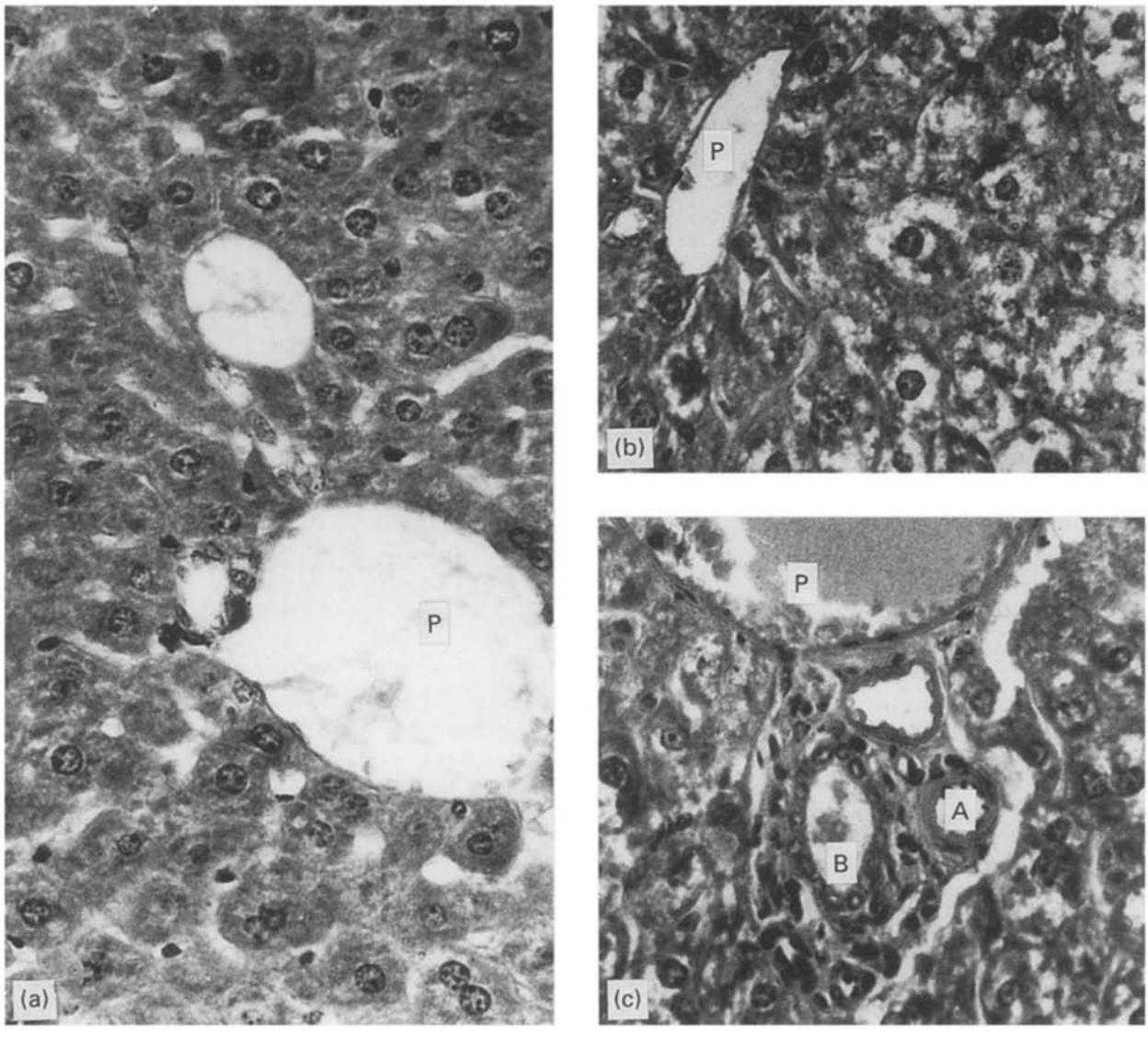


*Figure 1* (a) Light micrograph of the liver in a control mouse, where representative hepatocellular morphology is noted. (b, c) Light micrographs of the liver of stainless-steel-administered mice for 14 days, showing massive degeneration of the hepatocyte cords. Vocuolation of hepatocytes is evident, but nuclei display normal characterists. The typical arrays of hepatocytes are maintained, but cytoplasmic rarefaction is noted. Portal track shows apparently normal morphology. P – portal vein; A – arteriole; B – bile ductus. × 650.

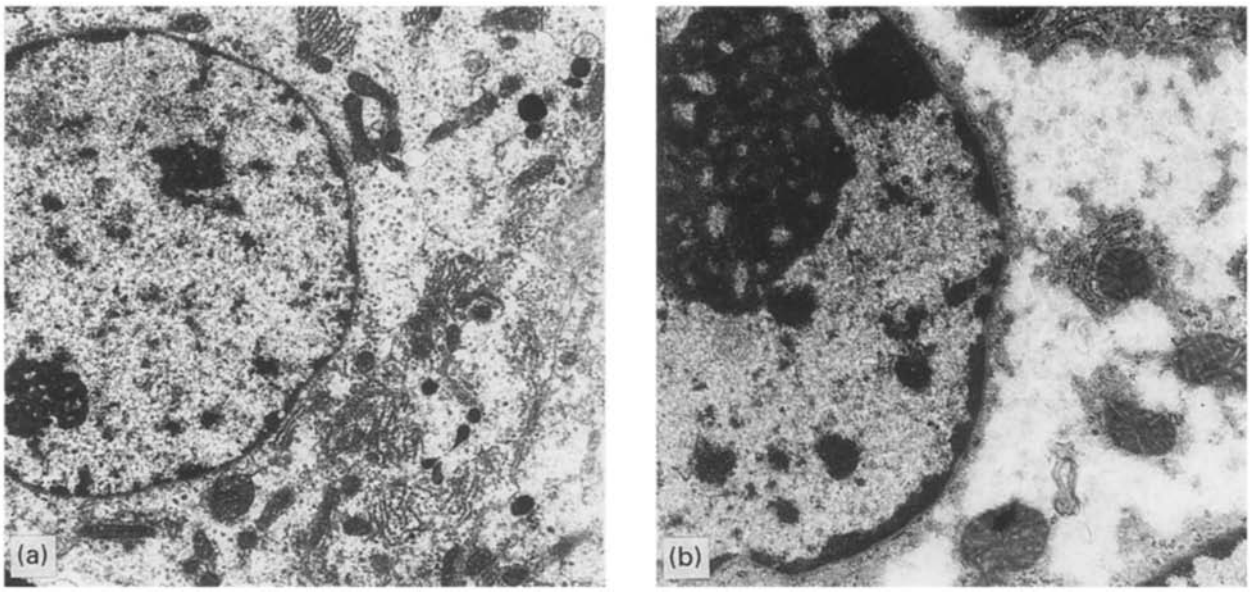


*Figure 2* (a) Electron micrograph of an hepatocyte in the control group. Some oval and round mitochondria surrounded by rough endoplasmic reticulum cisternae are denoted in the cytoplasm; × 10000. (b) Clear areas are seen in the cytoplasm, where cellular organelles are missing. The ultrastructural features of hepatocyte nucleus are normal. × 16000.

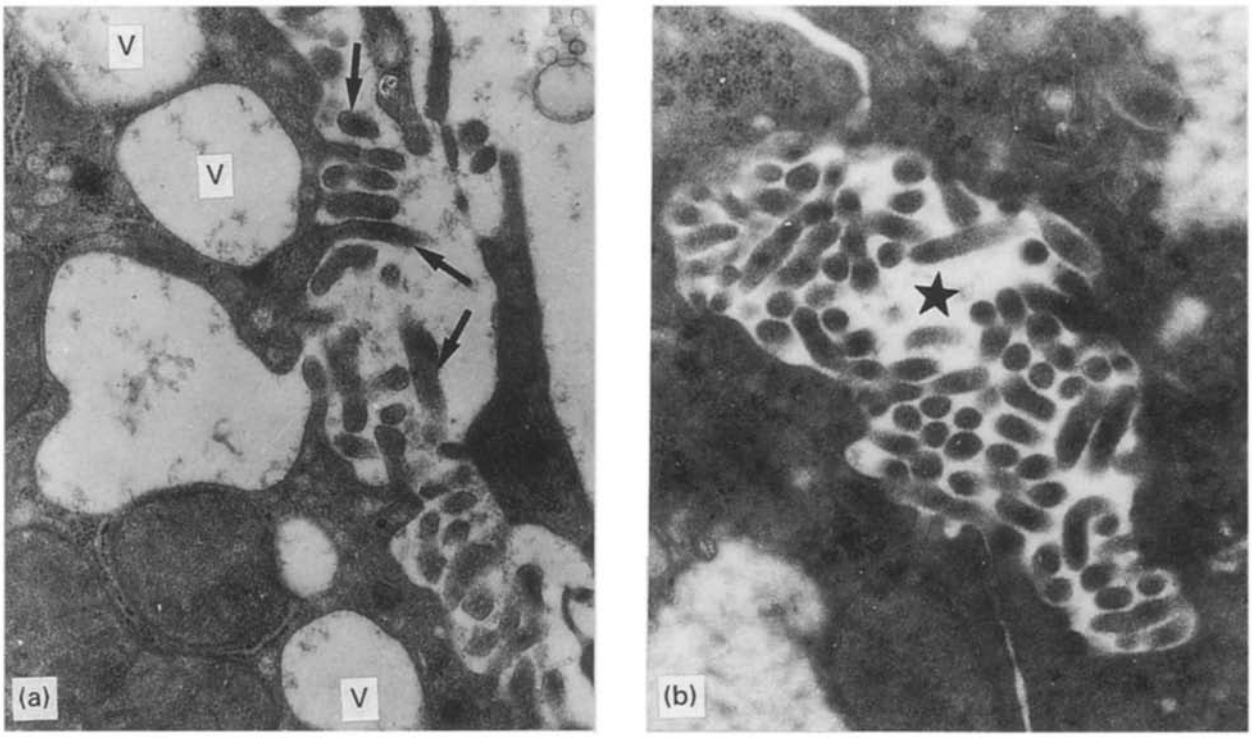


*Figure 3* Electron micrographs of mice liver 14 days after stainless steel administration. (a) Surface of hepatocyte is bordered by many microvilli (arrow); × 32000. (b) Bile canaliculi (✡) show normal ultrastructural morphology. H – hepatocyte; V – vacuoles. × 40000.

growth, however, the increase in liver wet weight was most probably due to the intake of water and other fluids, as the augmentation of hepatocyte vacuoles suggest. Of course, there must be a close association between the chromium deposition and the water intake, as the osmotic pressure caused by the metal ion concentration causes water influx, in the osmotic process, in order to contribute to the liver homeostasis.

Similarly, swollen hepatocytes have been reported in mice injected with perfluorodecanoid acid [19]. Furthermore, the massive liver degeneration associated with parenchymal cells observed in the present study resemble those previously described in experimentally induced chronic hepatitis mice [20].

Metallic corrosion products are transported through the body [16], are detected in blood cells and serum [11, 16, 21], are excreted in the urine [14, 16] and are accumulated in the spleen [16, 18]. Our present study confirms previous evidence that chromium accumulates in the liver [16] and further demonstrates that this metallic element induces not only increases in liver wet weight but also histological and ultrastructural alterations. Being a blood filtering organ, these liver morphological alterations constitute a matter of

some concern since the hepatocyte function may be hampered. Studies are now in progress in our laboratory in order to investigate the underlying mechanisms of stainless steel-associated hepatic injury.

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## References

1. O. E. M. POHLER, in "Biomaterials in reconstructive surgery" (C. V. Mosby, St. Louis, 1983) p. 158.
2. J. BLACK, E. C. MAINTIN, H. GELMAN and D. M., *Biomaterials* **4** (1983) 160.
3. A. BARTOLOZZI and J. BLACK, *ibid.* **6** (1985) 99.
4. J. V. LINDEN, S. M. HOPFER, H. R. GOSSLING and F. W. SUNDERMAN, *Ann. Clin. Lab. Sci.* **15** (1985) 459.
5. R. MICHEL, *CRC Critical Reviews in Biocompatibility* **3** (1987) 235.
6. H. F. HILDEBRAND, P. OSTAPCZUK, J. F. MERCIER, M. STOEPPLER, B. RONMAZEILLE and F. DECOULX, in "Biocompatibility of Co–Cr–Ni alloys" (Plenum Publishing Corporation, NATO-ASI Series A, Vol. 158, London, 1988) p. 133.
7. F. W. SUNDERMAN, S. M. HOPFER, Th SWIFT, W. N. REZUKE, L. ZIEBKA, P. HIGHMAN, B EDWARDS, M. FOLCIK and H. R. GOSSLING, *J. Orthop. Res.* **7** (1989) 307.
8. J. J. JACOBS, A. K. SKIPOR, J. BLACK, C. HASTINGS, J. SCHAVOCKY, R. M. URBAN and J. O. GALANTE, *Trans. Soc. Biomater.* **13** (1990) 199.
9. F. F. HENNIG, H. J. RAITHEL, K. H. SCHALLER and J. R. DÖHLER, *J. Trace Elem. Electrolytes Health Dis.* **6** (1992) 239.
10. J. WOODMAN, J. BLACK and S. JIMENEZ, *J. Biomed. Mater. Res.* **18** (1984) 99.
11. K. MERRIT, S. A. BROWN and N. A. SHARKEY, *ibid.* **18** (1984) 991.
12. K. L. WAPNER, D. M. MORRIS and J. BLACK, *ibid.* **20** (1986) 219.
13. S. A. BROWN, L. J. FARNSWORTH, K. MERRIT and T. D. CROWE, *ibid.* **22** (1988) 321.
14. K. MERRIT, T. D. CROWE and S. A. BROWN, *ibid.* **23** (1989) 845.
15. K. MERRIT, R. W. MARGEVICIUS, and S. A. BROWN, *ibid.* **26** (1992) 1503.
16. S. A. BROWN, K. ZHANG, K. MERRIT and J. H. PAYER, *ibid.* **27** (1993) 1007.
17. M. L. PEREIRA, A. SILVA, R. TRACANA and G. S. CARVALHO, *Cytobios* **77** (1994) 73.
18. R. B. TRACANA, M. L. PEREIRA, A. M. ABREU, J. P. SOUSA and G. S. CARVALHO, *J. Mater. Sci. Mater. Med.* **6** (1995) 56.
19. J. P. BONDE and J. M. CHRISTENSEN, *Arch. Environm. Health* **46** (1991) 225.
20. T.J. STINSON, S. JAW, E. H. JEFFREY and M. J. PLEWA, *Toxicol. Appl. Pharmacol.* **117** (1992) 98.
21. M. W. HARRIS, L. C URAIH and L. S. BIRNBAUM, *Fund. Appl. Toxicol.* **13** (1992) 723.

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