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Migration of diethylhexyl phthalate from PVC bags into intravenous cyclosporine solutions

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Abstract

The migration of diethylhexyl phthalate (DEHP) from PVC bags into LVPS (0.9% NaCl) and LVPS with cyclosporine at concentrations of 2.5 and 0.5 mg/ml was studied. PVC bags were placed in contact with these solutions and stored at 25 ± 1 °C. They were taken for analysis each 30 min during 6 h and after this period at each 1 h until 12 h of contact. Water was used as reference, and exposed and analyzed under the same conditions. After contact, the solutions were submitted to extraction with hexane and analyzed by GC–FID. The results showed that DEHP did not migrate into water and LVPS during all the time. Also, no measurable amount of DEHP was detected during the first 3 h of contact between the PVC bag and the diluted cyclosporine solution. However, the amount of released DEHP reached a detectable level after 4 h of contact, increased until 6 h, stabilized, and increased again after 9–10 h. The 12 h of contact showed the highest DEHP levels for both cyclosporine concentrations. The DEHP migrated was 0.02–0.08% of that present in the bag.

Keywords: Migration; Diethylhexyl phthalate; PVC bags; Cyclosporine; Intravenous solutions; Gas chromatography

1. Introduction

Cyclosporine is a potent immunosuppressive agent used mainly to prevent organ rejection following kidney, liver and heart transplantations. It has also been utilized alone or in combination with other medications to treat aplastic anemia, ulcerative colitis, psoriasis and rheumatoid arthritis [1,2]. Cyclosporine is characterized as a lipophilic cyclic polypeptide (Fig. 1). Because of cyclosporine's poor solubility in water, its current clinical formulations contain large amount of the non-ionic surfactant and polyoxyethylated castor oil (Cremophor® EL).

By intravenous administration, cyclosporine is directly and rapidly introduced into the blood circulation. This procedure requires vehicles such as large volume parenteral solutions (LVPS), which are commonly packed in plastic flasks or bags. Although glass and plastic containers are used for intravenous infusion of drugs, bags offer several advantages over conventional glass containers, like easier storage and shipping because of their relative resistance to breakage. However, several problems are associated with their use, such as the migration of potentially harmful chemicals into the solution [3–12], particularly diethyhexyl phthalate (DEHP), which is the main plasticizer used to produce PVC flexible bags. Due to DEHP hydrophobicity, it does not migrate or only low levels migrate into aqueous solution packed in PVC bags. But when surfactants such as Cremophor® EL or solvents as ethanol are used like formulation components large amounts of DEHP can migrate.

There is little information available regarding the use of cyclosporine and its interactions with plastic containers during intravenous infusion therapy. The manufacturers of cyclosporine recommend that for intravenous infusion the drug should be diluted in 0.9% NaCl injection or 5% glucose injection and administered slowly during 6 h. Furthermore, the

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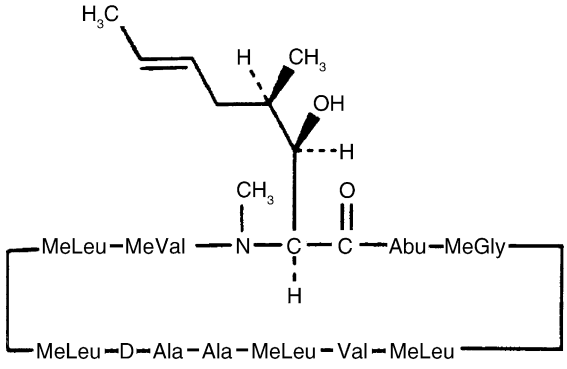

Fig. 1. Chemical structure of cyclosporine.

surfactant Cremophor® EL may allow DEHP to migrate from the polyvinyl chloride (PVC) surface [13]. It is recommend that intravenous fluids should be stored in glass containers and if PVC bags are used, these fluids should be used immediately after preparation to minimize patient exposure to DEHP and that the DEHP levels in the bags should be according to the European Pharmacopoeia [13].

Although DEHP within the human body has not been made clear yet, the intake of DEHP into human body through bags to LVPS should be avoided, as recommended by World Health Organization [14].

The aim of this work was to study the migration of DEHP from PVC flexible bags into LVPS (0.9% NaCl) and LVPS with cyclosporine solution.

2. Experimental

2.1. Chemicals, standards and bags

The hexane used was Merck (Darmstadt, Germany), HPLC grade. Acetone analytical reagent grade (Merck, Darmstadt, Germany) was used to rinse the glassware. Anhydrous sodium sulfate analytical reagent grade was from Mallinckrodt (Paris, France). Sodium chloride analytical reagent grade (Mallinckrodt, Paris, France) was used to prepare 0.9% NaCl solution. Di (2-ethylhexyl)phthalate (DEHP) 99.5% purity quality was purchased from Fluka (Buchs, Switzerland) and it was used without further purification.

Cyclosporine ampoules (5 ml), Sandimmun® concentrate for intravenous infusion 50 mg/ml, were from Novartis (Rio de Janeiro, Brazil).

PVC bags to LVPS were supplied by a Brazilian manufacturer of LVPS (São Paulo, Brazil), which contained 26 g DEHP/100 g bag, as previously determined in our laboratory.

2.2. Solutions

A 1000 μg/ml standard stock solution of DEHP in hexane was kept at −20 °C for no more than 1 month. Working hexane solutions were then prepared as needed. Calibration was performed with diluted working solutions in hexane (0.80–10 μg/ml).

LVPS (0.9% NaCl solution) was prepared in our laboratory. The cyclosporine was diluted with 0.9% NaCl solution, in an all-glass system, to yield a final concentration of 2.5 mg/ml (1:20 dilution) and 0.5 mg/ml (1:100 dilution).

2.3. Chromatographic conditions

Chromatographic analyses were carried out on a 17A Shimadzu Gas Chromatograph (GC) equipped with a flame ionization detector (FID). A DB-5 (J&W Scientific, Folsom, USA) capillary column of 60 m length × 0.25 mm i.d., 0.25 μm film thickness was used. The column temperature started at 100 °C, then programmed at 10 °C/min to 280 °C and held for 10 min. Hydrogen was the carrier gas and nitrogen was the make-up gas. Injections (1 μl) were made at 250 °C and the injector was operated in a splitless/split (1:20) mode, with sampling time of 30 s. Detector temperature was 300 °C. The time of retention for DEHP was 24.0 min.

2.4. PVC bags and LVPS analysis

Samples (1.0 g) of PVC plastic bags ($n = 10$) were manually cut into 1 cm^2 pieces and extracted with 20.0 ml of acetone in ultrasonic bath for 12 min. A 10.0 ml aliquot was removed from the acetone extract and the solvent was evaporated under nitrogen stream. The residue was reconstituted with hexane (50.0 ml) and the solution obtained after adequate dilution, was analyzed by GC–FID. The chromatogram obtained from PVC bag extracts showed only one peak, identified as DEHP.

5.00 ml LVPS with and without cyclosporine were extracted with 10.0 ml hexane under sonication for 12 min. Afterwards, this mixture was allowed to stand for 15 min. After this period, the organic phase was separated using a volumetric pipette (5.0 ml) and anhydrous sodium sulfate was added. Aliquots of 1 μl of the liquid supernatant were analyzed by GC–FID.

2.5. Migration study

The sections of PVC bags were cut into pieces of 6 cm^2 (2 cm × 3 cm) surface area, which weighed 0.20–0.25 g each. Each piece was placed in a 20 ml glass vial containing 10 ml of 0.9% NaCl or 10 ml of 0.9% NaCl with cyclosporine at concentrations of 2.5 and 0.5 mg/ml. The vials were hermetically capped and stored in an oven thermostatically set at 25 ± 1 °C. The sample vials were analyzed each 30 min during the first 6 h of the experiment, thereafter sample vials were analyzed every hour until 12 h. The PVC sections were discharged prior to extraction. The migration study was carried out using three sample vials of each studied solution, at each period of time. A blank prepared only with water was used as reference, and exposed and analyzed under the

same conditions. All migration tests were carried out by total immersion.

2.6. Quantitation

For quantitation peak area was measured and external standard procedure was used for both calibration and real sample analysis. The limit of detection for DEHP was 0.40 ng/ml and the limit of quantitation was 0.80 μg/ml.

The DEHP migration levels obtained were submitted to the ANOVA. Tukey test was used to compare the difference among means at $p \leq 0.05$.

3. Results and discussion

The migration study was carried out using 0.9% NaCl solution, one of the vehicles that should be used for the intravenous administration of the cyclosporine. The Sandimmun® manufacturers recommend that it should be diluted at 1:20 to 1:100 in 0.9% NaCl injection or 5% glucose injection and also that the intravenous solution must be infused slowly during 2–6 h [13]. In this work the limit dilutions (1:20 and 1:100) were studied, as recommended. It was carried out according to the official migration tests [15–17] for plastic materials intended to come into contact with foodstuffs, since no official migration tests exist for plastic drug containers. The migration study was carried out at 25 ± 1 °C in order to simulate the conditions routinely used in the clinical practice. The contact time of 6 h [13] was extended until 12 h to study the migration behavior.

The GC–FID analysis showed that no detectable amount of DEHP migrated into water (blank test) or 0.9% NaCl during the time frame of the investigation. In accordance with our results, Dine et al. [18] did not also detected DEHP in water, 0.9% NaCl or 5% glucose stored in plastic bags for 1 year. Otherwise, Arbin and Östelius [19] quantified mono 2-ethylhexyl phthalate and DEHP in 5% glucose and 0.9% NaCl stored in PVC bags during 15 days to 14 months. The amount of DEHP migrated into these solutions varied from 4 to 34 ng/ml, but a more sensitive detector (electron capture detector) was used.

The DEHP was not detected in 0.9% NaCl solutions with cyclosporine until 3 h of contact, at any dilutions (1:20 and 1:100). After 3 h of contact the DEHP was detected but not quantified since the amount obtained were lower than the limit of quantitation of the method. Nevertheless from 3.5 and 4 h of contact for 1:20 and 1:100 dilutions, respectively, the DEHP could be quantified. Migration curves (DEHP concentration × time of contact) were obtained for both dilutions (Fig. 2) using a third order polynomial regression $y = a + b_1x + b_2x^2 + b_3x^3$.

The kinetics of the migration process can be observed in both sinusoidal curves indicating the same tendency of DEHP migration into LVPS at both dilutions (Fig. 2). The migration rates rapidly increase until 6 h of contact and after this period they were stabilized. However, after 10 h of contact the migration rate rapidly increased again showing a more pronounced increase for 1:20 dilution than for 1:100 dilution. This same migration tendency was previously described by Monteiro et al. [20] when migration curves for UV stabilizer from polyethyleneterephthalate (PET) bottles into fatty-food simulants were obtained.

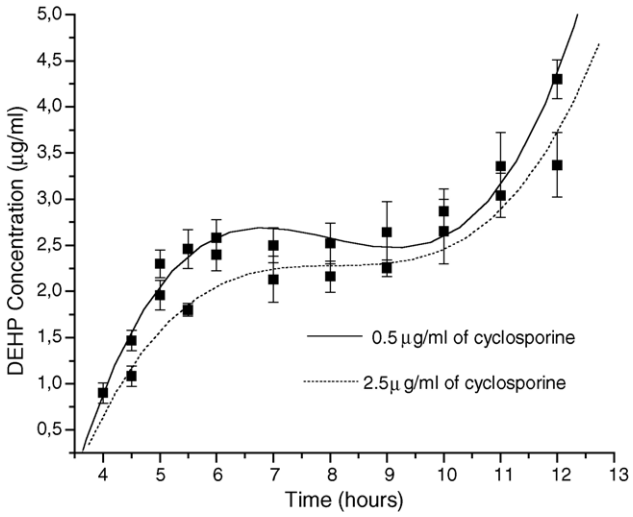

Fig. 2. Migration curve of DEHP from PVC bags into LVPS (0.9% NaCl) with cyclosporine at 1:20 and 1:100 dilutions.

Since this migration study employed the limit dilutions recommended by the cyclosporine manufacturer, our results may represent the minimum and the maximum levels of DEHP that will migrate when the drug is infused into patients. The migration curves results suggest that LVPS with cyclosporine could be used until 11 h (Fig. 2). No significant difference ($p > 0.05$) was found for DEHP migrated levels between 5.5–11 h and 6–11 h of contact for LVPS with cyclosporine at 1:20 and 1:100 dilutions, respectively (Table 1). The 12 h of contact showed the highest DEHP levels (4.30 and 3.37 μg/ml), differing significantly ($p \leq 0.05$) from the

Table 1
Migration level of DEHP (μg/ml) from PVC bags into 0.9% NaCl with cyclosporine at 1:20 and 1:100 dilutions at different contact times

Time (h)	Migration level of DEHP (μg/ml; dilutions[1] of cyclosporine in 0.9% NaCl)	
	1:20	1:100
4.0	0.90 ± 0.11 e	–
4.5	1.47 ± 0.11 d,e	1.08 ± 0.11 d
5.0	2.30 ± 0.15 c,d	1.96 ± 0.16 c,d
5.5	2.46 ± 0.21 c,b	1.80 ± 0.07 c,d
6.0	2.58 ± 0.20 b	2.40 ± 0.18 b,c
7.0	2.50 ± 0.19 b	2.13 ± 0.25 c
8.0	2.52 ± 0.22 b	2.16 ± 0.17 b,c
9.0	2.64 ± 0.33 b	2.25 ± 0.09 b,c
10.0	2.65 ± 0.35 b	2.87 ± 0.24 b
11.0	3.36 ± 0.36 b	3.04 ± 0.24 a,b
12.0	4.30 ± 0.58 a	3.37 ± 0.35 a

Mean ± S.D. value for three replicates, $n = 3$. Means within the same row followed by like letters are not significantly different ($p < 0.05$).

other periods for the 1:20 dilution while there was no significant difference ($p > 0.05$) between 11 h of contact for 1:100 dilution. The migration level of DEHP was 0.02–0.08% of that found in the bag.

Several migration studies have been reported for DEHP from PVC bags into LVPS with drugs [3–12]. These studies were carried out using two kinds of assay: the simulated infusion and the storage. The first kind comprised quantitation of DEHP migrated into LVPS with the drug after specific contact times while the infusion was simulated. In this procedure, an infusion pump controlled the flow and aliquots of the effluent were collected at each time and analyzed. In the storage assay, the LVPS with the drug was stored in the bag, under controlled conditions of time and temperature, similarly to our work.

DEHP migration from PVC bags containing 5% glucose and cyclosporine was reported by Venkataramanan et al. [9] who determined 1.7, 18, 69 and 130 μg/ml of DEHP after 0, 2, 4 and 8 h of contact, respectively. Our migration results showed DEHP concentration lower than those observed by Venkataramanan et al. [9]. This could be probably related to the cyclosprine concentration, which may have been higher than in our study.

Waugh et al. [10] found high amount of DEHP (150 and 340 μg/ml, after 9 and 25 h of contact, respectively) migrated from PVC bags into solutions containing paclitaxel formulation during a simulated infusion assay. The authors suggested that the migration of DEHP was due to the large percentage of Cremophor® EL present in the pharmaceutical formulation. Faouzi et al. [5] determined the migration of DEHP from PVC bags into 0.9% NaCl and 5% glucose containing teniposide after 48 h of storage. They also collected aliquots at different periods of time during 1 h of simulated infusion. They reported that the DEHP migrated into intravenous solutions over 48 h at room temperature was 52 and 19 mg at 4 °C. In the simulated infusion assay the DEHP migrated levels varied from 0 to 10 μg/ml, progressively increasing until 1 h of infusion. Other similar migration study was described by Faouzi et al. [6] who determined DEHP migrated from PVC bags into 5% glucose and 0.9% NaCl solutions with miconazole. Aliquots were withdrawn and analyzed after 2 h of simulated infusion. The storage assay has been also studied with the solutions stored in PVC bags for 24 h at 4 °C and at room temperature. They found 1.35 and 0.64 mg of DEHP in the 5% glucose and 0.9% NaCl solutions with miconazole after simulated infusion and storage, respectively. Allwood and Martin [3] determined the DEHP migration from PVC bags into 5% glucose containing paclitaxel at two dilutions (5 and 10%, v/v). The study comprised two kinds of assay: a simulated infusion during 3 h, in which aliquots were collected and analyzed for the determination of DEHP, and the storage of the preparations in the bags for 3 h followed by the simulated infusion procedure. The amount of DEHP migrated into 5% glucose containing paclitaxel at 5 and 10% dilutions after 3 h of infusion was 15.45 and 44.30 mg, respectively. When the paclitaxel was stored for 3 h followed by infusion, the amount of DEHP migrated into solution at 5 and 10% after the simulated infusion were 27.4 and 70.0 mg, respectively [3]. To evaluate the potential risks of the exposure of patients to DEHP Faouzi et al. [7] studied the DEHP migration into 5% glucose containing quinine stored in PVC bags and they also analyzed the preparation during a simulated infusion for 2 h. The storage times were 48 and 72 h at 4 °C and 8 h at room temperature. When the preparation was stored at 4 °C for 4 h, the DEHP migrated was 4.5 μg/ml, whereas the storage at room temperature for 8 h yielded DEHP concentrations lower than 5.5 μg/ml. The amount of DEHP migrated after 2 h infusion collected at the end of the administration sets was lower than 2 μg/ml.

The amount of DEHP obtained in this migration study was lower, in all the periods of time studied, than those described by Venkataramanan et al. [9], Waugh et al. [10], Pearson and Trissel [11], Faouzi et al. [5–7], and Allwood and Martin [3] who studied the migration of DEHP from PVC bags into LVPS with drugs. This fact can be attributed to the different procedures and conditions used in the migration studies. On the other hand, in the present work the amount of DEHP in the bags was quantified previously to the migration study differently from the other authors [3–12]. They did not determined DEHP in the bags, suggesting that the amount of DEHP in the bags was substantially higher than those found in the PVC bags used in our study.

Venkataramanan et al. [9] observed the migration of DEHP into 5% glucose containing only the vehicles (ethanol and Cremophor® EL) present in cyclosporine solution for intravenous infusion. The concentration of DEHP in the solution containing the vehicles alone, after 8 h of contact, was 116 μg/ml which was similar to that migrated into 5% glucose containing cyclosporine (130 μg/ml). Pearson and Trissel [11] evaluated the migration of DEHP from PVC bags into several organic solvents and surfactants used as formulation components and into 12 drugs products containing these solvents and surfactants. The organic solvents (ethanol, polyethylene glycol and propylene glycol), the surfactants (polysorbate 80 and polyoxyethylated castor oil) and 12 drugs were diluted separately with 5% glucose stored in PVC bags. The highest concentrations of DEHP, 46.6, 81.1 and 197.8 μg/ml, after 4, 8 and 24 h of contact, respectively, migrated into the mixture of 5% polyoxyethylated castor oil and 5% ethanol. Among the studied drugs, the highest concentration of DEHP was found in the cyclosporine solution: 10.2, 27.2, 98.8 μg/ml, after 4, 8 and 24 h of contact, respectively. It should be noticed that the concentration of surfactant in the LVPS after cyclosporine final dilution was 3.6%. Allwood and Martin [3] also verified the DEHP migration into 5% glucose containing only the vehicle of the pharmaceutical formulation (Cremophor® EL and ethanol). The amount of DEHP migrated after 3 h of infusion were 15.45 and 44.30 mg for 5 and 10% dilutions, respectively. These results were close to those obtained from 5% glucose solution containing paclitaxel, showing that the presence of the drug alone did not influence the amount of DEHP migrated.

This work has not verified the migration of DEHP into LVPS containing only the surfactant used in the cyclosporine formulation.

Several studies have shown the toxicity of DEHP on laboratory animals mainly during exposure to diet. Some of them have associated DEHP with hepatotoxic effects in rats [21–24]. Poon et al. [25] described histological changes in the thyroid of rats and Arcadi et al. [26] observed changes in the body weight gained by offspring rats after perinatal exposure to DEHP and histological damages in kidneys, liver and testes of rats. Testicular atrophy has been the main reproductive effect in animals associated with the exposure to DEHP [14]. The carcinogenicity of DEHP in rats has been showed in several studies [27,28]. Nevertheless, little information is available on the effects of DEHP on humans [14]. DEHP appears to pose a relatively low risk of hepatic cancer in humans. However, given lingering uncertainties about the relevance of the mechanism of action of carcinogenic effects in rodents for humans and interindividual variability, the possibility of DEHP-related carcinogenic responses in humans can not be ruled out [29].

4. Conclusions

The migration study revealed that the DEHP migrated from PVC bags into 0.9% NaCl containing cyclosporine at concentrations of 2.5 and 0.5 mg/ml; DEHP migration levels could be quantified after 4.5 and 5 h of contact, respectively. DEHP migration into LVPS containing the drug at two concentrations showed the same behavior. The highest levels of DEHP were obtained in the LVPS with cyclosporine for both concentrations at 12 h of contact.

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References

[1] B.D. Kahan, Med. Intell. 321 (1989) 1725–1738.
[2] D.B. Sachar, N. Engl. J. Med. 321 (1989) 894–896.
[3] M.C. Allwood, H. Martin, Int. J. Pharm. 127 (1996) 65–71.
[4] A.P. Cheung, Y.F. Hallock, B.R. Vishnuvajjala, T. Nguyenle, E. Wang, Invest. New Drugs 16 (1998) 227–236.
[5] M.A. Faouzi, M. Luyckx, B. Gressier, F. Goudaliez, M.L. Mallevais, C. Brunet, M. Cazin, J.C. Cazin, Int. J. Pharm. 105 (1994) 89–93.
[6] M.A. Faouzi, T. Dine, M. Luyckx, C. Brunet, M.L. Mallevais, F. Goudaliez, B. Gressier, M. Cazin, J. Kablan, J.C. Cazin, J. Pharm. Biomed. Anal. 13 (1995) 1363–1372.
[7] M.A. Faouzi, F. Khalfi, T. Dine, M. Luyckx, C. Brunet, B. Gressier, F. Goudaliez, M. Cazin, J. Kablan, A. Belabed, J.C. Cazin, J. Pharm. Biomed. Anal. 21 (1999) 923–930.
[8] B. Mass, C. Huber, I. Kramer, Pharm. World Sci. 18 (1996) 78–82.
[9] R. Venkataramanan, G.J. Burckart, R.J. Ptachcinski, R. Blaha, L.W. Logue, A. Bahnson, C.S. Giam, J.E. Brady, Am. J. Hosp. Pharm. 43 (1986) 2800–2802.
[10] W.N. Waugh, L.A. TrisseL, V.J. Stella, Am. J. Hosp. Pharm. 48 (1991) 1520–1524.
[11] S.D. Pearson, L.A. Trissel, Am. J. Hosp. Pharm. 50 (1993) 1405–1409.
[12] S.B. Craig, U.H. Bhatt, K. Patel, J. Biomed. Anal. 16 (1997) 199–205.
[13] A.G. Novartis, Sandimmun Package Insert, Basiléia, Suiça, 1996.
[14] World Health Organization, Diethylhexyl Phthalate (Environmental Health Criteria 131), Geneva, 1992, 141 pp.
[15] EC, Council Directive 82/711/EEC of 18 October, laying down the basic rules necessary for testing migration of the constituents of plastic materials and articles intended to come into contact with foodstuffs, Off. J. Eur. Commun. L372 (1985) 14–21.
[16] Mercosul, General Regulations for Plastic Packaging and Equipment Intended to be in Contact with Food: Conditions for the Migration Test, Resolução 36/92 do Grupo Mercado Comum, Montevidéo, 1995.
[17] Sanity Agency/Ministry of Health, Brazil, Resolução no. 105, de 19 de maio de 1999, Technical Regulation – General Regulations for Plastic Packaging and Equipment Intended to be in Contact with Food, Diário Oficial, Brasília, 20 de maio de 1999.
[18] T. Dine, M. Luychx, M. Cazin, C. Brunet, J.C. Cazin, Biomed. Chromatogr. 5 (1991) 94–97.
[19] A. Arbin, J. Östelius, J. Chromatogr. 193 (1980) 405–412.
[20] M. Monteiro, C. Nerín, F.G.R. Reyes, Packaging Technol. 12 (1999) 241–249.
[21] E.D. Barber, B.D. Astill, E.J. Moram, B.F. Schneider, T.J.B. Gray, B.G. Lake, J.G. Evans, Toxicol. Ind. Health 3 (1987) 7–24.
[22] P.K. Eagon, N. Chandan, M.J. Epley, M.S. Elm, E.P. Brady, K.N. Rao, Int. J. Cancer 58 (1994) 736–743.
[23] L.D. Winberg, M.Z. Badr, Toxicol. Lett. 76 (1995) 63–69.
[24] T. Yanagita, M. Satoh, N. Enomoto, N. Suganom, Biochim. Biophys. Acta 919 (1987) 64–70.
[25] R. Poon, P. Lecavalier, R. Mueller, V.E. Valli, B.G. Procter, I. Chu, Food Chem. Toxicol. 35 (1997) 225–239.
[26] F.A. Arcadi, C. Costa, C. Imperatore, A. Marchese, A. Rapisarda, M. Salemi, G.R. Trimarchi, G. Costa, Food Chem. Toxicol. 36 (1998) 963–970.
[27] M.S. Rao, A.V. Yeldandi, V.J. Subbarao, Toxicol. Environ. Health 30 (1990) 85–89.
[28] J.A. Popp, L.K. Garvey, R.C. Cattley, Toxicol. Ind. Health 3 (1987) 151–163.
[29] J.A. Tickner, T. Schettler, T. Guidotti, M. McCally, M. Rossi, Am. J. Ind. Med. 39 (2001) 100–111.