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THE RENAL FRACTIONAL CLEARANCE OF PLATINUM ANTITUMOUR COMPOUNDS IN RELATION TO NEPHROTOXICITY

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Abstract—The fractional renal clearance of platinum relative to inulin was measured in the conscious rat during the period 60–70 min after injection of the nephrotoxic drug, cisplatin, or its non-nephrotoxic analogues (*trans*-dichlorodiammine platinum II, carboplatin or iproplatin). The fractional clearance of platinum was between 3 and 4 for cisplatin and its analogues. Platinum from cisplatin and its trans isomer is essentially irreversibly bound whilst that from carboplatin and iproplatin is largely reversibly bound to blood proteins. Probenecid and triethanolamine both caused an increase whereas furosemide caused a decrease in the fractional clearance of total platinum from cisplatin. Choline chloride had no nett effect on the fractional clearance of total platinum. Both furosemide and triethanolamine made no significant difference to the severity of cisplatin induced nephrotoxicity. However, probenecid enhanced cisplatin induced nephrotoxicity and choline chloride was capable of blocking cisplatin induced nephrotoxicity. We conclude that the renal tubular transport of platinum is not *per se* responsible for the nephrotoxicity of platinum compounds. However, in the case of cisplatin, or one of its metabolites, renal tubular transport may be a prerequisite for nephrotoxicity.

Cisplatin (*cis*-dichlorodiammine platinum II) is a widely used antitumour drug [1] with severe nephrotoxic side-effects [2]. There are structural analogues of cisplatin which do not exhibit nephrotoxicity, several of which are currently on clinical trial [1].

We have reported previously that cisplatin and its metabolites are excreted in part by renal tubular transport. This was established by measurement of the renal clearance of platinum relative to inulin (fractional clearance) in whole rats [3, 4] and in the isolated perfused rat kidney [5]. Renal secretion of platinum has also been described in humans treated with cisplatin [6]. This phenomenon may be important in the development of the platinum-induced renal lesion since the process probably involves the concentration of platinum in kidney tubular cells. In this study we have investigated further the renal tubular transport of cisplatin and compared the renal fractional clearance of non-nephrotoxic analogues with that of cisplatin. We have also investigated the effect of competitive inhibitors of different renal tubular transport mechanisms on the fractional clearance and nephrotoxicity of cisplatin.

MATERIALS AND METHODS

Chemicals. Sources: Platinum compounds; cisplatin (*cis*-dichlorodiammine platinum II), *trans*-DDP (*trans*-dichlorodiammine platinum II), carboplatin (diammine 1,1-cyclobutanedicarboxylato platinum II), iproplatin [*cis*-dichloro-*trans*-dihydroxo-*cis*-bis(isopropylamine) platinum IV] were gifts from Johnson Matthey Research, Reading. Probenecid (Sigma Chemical Co., Poole), furosemide (DDSA Pharmaceuticals Ltd., London), triethanolamine–HCl, choline chloride and inulin (BDH Chemicals Ltd., Poole). All agents were administered to animals by the i.p. route as freshly prepared solutions in 0.9% NaCl.

Animals. All animals used in these experiments were male Wistar rats (Charles River, Margate), 350–450 g. Animals were housed in plastic cages and allowed *ad libitum* access to food and water.

Cannulation of rat ureters. Fractional renal clearance measurements were made in conscious animals prepared by surgical cannulation of the ureters. Animals were anaesthetised with sodium pentobarbital (50 mg/kg, i.p.) and plastic cannulae (size 3, intravenous, 15–20 cm in length, Portex, Kent) were inserted and sutured into the ureters of both kidneys. The cannulae were then led subcutaneously to exit at the back of the neck. The volume of the cannula tubing was 18 mm^3 and the approximate volume of urine collected in 10 min was 200 mm^3. Rats were allowed to recover for 24 hr after the operation before clearance measurements were made. The urine was collected, during the recovery period, in a glass saddle which was sutured to the skin on the back of the animal. During the experimental period urine was collected into preweighed plastic vials. For blood sampling the animals were restrained and blood (up to 0.5 cm^3) removed from the caudal vein.

Assay of platinum and inulin in urine and plasma samples. Both platinum and inulin were determined in urine and protein free plasma (pfp), platinum was also determined in whole plasma. Pfp was obtained from heparinized blood which was centrifuged to remove cells and then filtered through Amicon CF25 ultrafilters to remove proteins. Platinum was measured by flameless atomic absorption spectroscopy

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using a Perkin Elmer 2380 spectrophotometer with HGA400 graphite furnace and temperature controller. Inulin was measured using the method of Heyrovsky [7]. Where necessary samples were diluted with 0.9% NaCl before analysis.

Assessment of nephrotoxicity. Nephrotoxicity was assessed by measurements of blood urea (BUN) and by histopathological examination of the kidneys, as previously described [8].

Equilibrium dialysis. A heparinized syringe was used to collect blood by cardiac puncture from rats anaesthetized with ether. Plasma was prepared by centrifugation of freshly obtained blood. For each experiment a 1 cm^3 sample of blood or plasma was placed in viscose dialysis tubing (6.3 mm dia., Medicell, London). The sample was then suspended in a 50 cm^3 measuring cylinder containing 0.9% w/v NaCl (50 cm^3) and the platinum compound (cisplatin, *trans*-DDP, carboplatin, or iproplatin) at a concentration of approx. 10 $\mu g/cm^3$. The solutions were then allowed to reach equilibrium with constant stirring. In a preliminary experiment this was found to take 16 hr at room temperature. After this time samples of blood, plasma and saline were removed and assayed for platinum. The samples of blood and plasma were then transferred in their dialysis tubing to a fresh 50 cm^3 solution of 0.9% NaCl not containing platinum. Samples of the blood and plasma were then removed after various times during the second dialysis and the platinum concentration determined.

RESULTS

Fractional clearance measurements

Animals with cannulated ureters were hydrated with 0.9% NaCl (5.0 cm^3/kg) p.o., and then immediately injected with inulin (60 mg/kg) and the platinum compound (cisplatin, *trans*-DDP, carboplatin or iproplatin; 15 mg/kg). After 60 min urine was collected for 10 min. At the mid point of the urine collection period a blood sample was obtained. These samples were assayed for platinum and inulin. The clearance of platinum (C_{Pt}) and inulin (glomerular filtration rate, GFR) were calculated from the formula $U_x/P_x \times V$, where U_x and P_x are the urine and plasma concentrations of the substance and V is the urine flow rate in cm^3/min. The concentration of platinum in pfp (non protein-bound platinum) was used for P_x. The fractional clearance of platinum was calculated as follows: C_{Pt}/GFR. The results are shown in Table 1.

In one experiment, after dosing with cisplatin and inulin as described above, three successive fractional clearance measurements were made (during 55–60, 60–65 and 65–70 min after dosing). The results (Table 2) indicate that the plasma levels of platinum and inulin do not decline substantially during the time period encompassing the clearance measurements.

Protein binding studies

The binding of platinum from cisplatin, *trans*-DDP, carboplatin and iproplatin to blood proteins under equilibrium dialysis conditions was determined as described in the methods. The results from

Table 1. Renal fractional clearance and plasma distribution of platinum compounds in the rat 60–70 min after treatment

Compound (N = 6)	Platinum concentration ($\mu g/cm^3$)			Clearance (cm^3/min)		Plasma protein bound platinum (%)	Fractional clearance of total platinum (C_{Pt}/C_{inulin})
	Whole plasma	Protein free plasma	Urine	Platinum	Inulin		
Cisplatin	7.3 ± 3.1	3.2 ± 1.4	645 ± 428	3.6 ± 1.9	1.2 ± 0.8	54.0	3.08 ± 0.77
Trans-DDP	17.6 ± 3.7**	1.3 ± 0.5**	224 ± 132*	2.5 ± 2.0	0.79 ± 0.43	90.1**	3.28 ± 1.45
Carboplatin	7.7 ± 1.9	6.4 ± 1.4**	824 ± 330	2.7 ± 1.2	0.92 ± 0.4	16.9**	3.35 ± 1.56
Iproplatin	7.4 ± 2.4	3.9 ± 1.0	838 ± 843	3.9 ± 1.7	1.04 ± 0.47	47.5	4.04 ± 1.43

Significantly different from cisplatin treatment, **$P < 0.01$, *$P < 0.05$. Values are means ± S.D.

Table 2. Consecutive fractional clearance measurements made in a single rat during the period 55–70 min following injection of cisplatin and inulin

Time (min)	Platinum (μg/cm^3)		Inulin (μg/cm^3)		Fractional clearance (C_{Pt}/C_{inulin})
	Protein free plasma	Urine	Protein free plasma	Urine	
55–60	2.73	860	8.7	920	2.94
60–65	2.67	832	8.2	947	2.81
65–70	2.60	810	8.2	1068	2.31

plasma binding are shown in Fig. 1 and Table 3. When the experiment was repeated with whole blood the results were almost identical except that the amount of platinum bound at the end of the first dialysis was approx. 30% lower than the values shown for plasma.

The modification of renal transport of cisplatin by organic anions and cations

Animals with cannulated ureters were dosed with probenecid (200 mg/kg), furosemide (200 mg/kg), triethanolamine–HCl (200 mg/kg) or choline chloride (100 mg/kg). After 0.5 hr animals were dosed with cisplatin (15 mg/kg) and inulin (60 mg/kg) as described above. Fractional clearance measurements were then made as before, 60 min after the administration of cisplatin. The results are shown in Table 4.

The modification of renal toxicity of cisplatin by organic anions and cations

To test the effect of the organic anions and cations on cisplatin induced nephrotoxicity intact animals were dosed with probenecid (100 mg/kg), furosemide (100 mg/kg), triethanolamine–HCl (100 mg/kg) or choline chloride (30 mg/kg). After 30 min animals were dosed with cisplatin (5 mg/kg). Blood urea measurements were made daily for seven days. For all animals where BUN levels were elevated the maximum value was found on the fifth day after treatment. Kidneys were removed from treated animals on the fifth day after treatment for histopathological examination. In the damaged kidney the lesions were confined to the pars recta of the proximal tubule, and the severity of the damage was graded on a scale form − (no visible damage) to ++ (severe damage). The results are shown in Table 5.

DISCUSSION

Fractional clearance of cisplatin

The data presented here (Tables 1 and 2) agree with our earlier observations that platinum from cisplatin is secreted by the kidney of the conscious rat with a fractional clearance, relative to inulin, of approx. three [3]. Using the isolated perfused rat kidney we obtained a fractional clearance for cisplatin of 1.25–1.29. The lower values for the perfused kidney may be the result of the high perfusate flow rate employed in this *in vitro* preparation [5]. Osman and Litterst [9] reported a fractional clearance of 1.15 for platinum from cisplatin in the anaesthetized rat. In their experiments a continuous i.v. infusion of cisplatin and inulin was used. They did not report

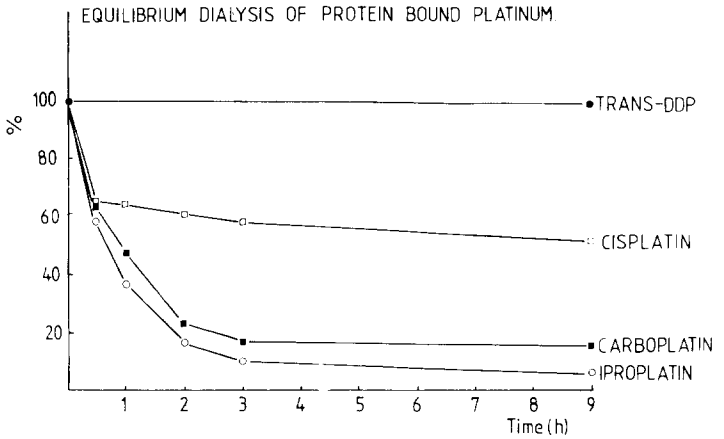

Fig. 1. Rat plasma was dialysed for 16 hr against various platinum compounds (10 μg/cm^3) dissolved in 0.9% NaCl. The figure shows the result of a second dialysis of the plasma against 0.9% NaCl, which was performed to assess the reversibility of the platinum–protein complexes that formed during the first dialysis. The decline with time of the total platinum concentration inside the dialysis bag is shown. The concentration of platinum both free and protein bound in the plasma, inside the dialysis bag at the beginning and end of the first and second dialysis is shown in Table 3.

Table 3. Equilibrium dialysis of plasma protein bound platinum compounds

Compound	Platinum concentration $\mu g/cm^3$)						Irreversibly† protein bound Pt (%)
	First dialysis			Second dialysis			
	0 hr saline	16 hr saline	16 hr plasma*	0 hr saline	9 hr saline	9 hr plasma*	
Trans-DDP	8.1	3.92	131.2 (97.0)	0.0	0.02	130.9 (99.9)	100.0
Cisplatin	10.66	8.66	39.92 (78.3)	0.0	0.38	20.64 (98.2)	64.8
Carboplatin	11.98	8.42	18.96 (55.6)	0.0	0.31	3.04 (89.8)	25.9
Iproplatin	6.9	4.54	11.84 (61.7)	0.0	0.22	0.72 (69.5)	6.8

* In parentheses: percentage of platinum inside dialysis bag which is protein bound. Values are means of three determinations.
† Percentage of platinum protein bound after first dialysis which is still bound after the second dialysis.

Table 4. The renal fractional clearance and plasma distribution of platinum 1 hr following treatment of rats with cisplatin and organic anions and cations

Treatment (N = 6)	Platinum concentration ($\mu g/cm^3$)			Plasma protein bound platinum (%)	Fractional clearance of total platinum (C_{Pt}/C_{inulin})
	Whole plasma	Protein free plasma	Urine		
Cisplatin + probenecid	11.3 ± 4.3	5.3 ± 3.3	1113 ± 318**	52.2	5.05 ± 1.39**
Cisplatin + furosemide	8.4 ± 4.1	3.1 ± 1.6	60 ± 39**	63.0	2.07 ± 0.77**
Cisplatin + triethanolamine HCl	8.2 ± 1.9	3.5 ± 0.8	406 ± 71	57.3	4.69 ± 1.7*
Cisplatin + choline chloride	6.6 ± 0.8	3.4 ± 0.4	502 ± 249	51.0	3.89 ± 1.27

Significantly different from cisplatin treatment (cf. Table 1). **$P < 0.01$, *$P < 0.05$. Values are means ± S.D.

Table 5. The nephrotoxicity of different platinum treatments in the rat

Treatment	N	BUN Day 5 (mg/dl)	Dose Pt excreted day 1 (%)	Histopathological damage
Control	15	20.2 ± 6.4		–
Cisplatin	9	95.6 ± 46.8	80.5	++
Cisplatin + probenecid	6	152.2 ± 30.5*	74.8	++
Cisplatin + furosemide	4	108.5 ± 42.0	49.2	N.D.
Cisplatin + triethanolamine HCl	4	114.0 ± 45.3	64.0	++
Cisplatin + choline chloride	6	36.7 ± 9.2**	42.0	+
Trans-DDP	4	27.1 ± 3.2†	76.6	–

N.D., no data.
† Not significantly different from the control, $P < 0.01$.
* Significantly different from cisplatin treatment, $P < 0.05$.
** Significantly different from cisplatin treatment, $P < 0.01$.
Values are means ± S.D.

whether steady state plasma levels of platinum were achieved by this method. However, anaesthesia is usually accompanied by a fall in GFR and this would result in low urinary output and an increase in the tubular modification of the formative urine. Siddik *et al.* [10] reported a renal clearance value for cisplatin in the rat of 1.28 times the clearance of inulin; however, their protocol is not described.

We have established that following a single i.p. injection of cisplatin (15 mg/kg) and inulin (60 mg/kg) there is a decline of only 4–5% in the plasma levels of platinum and inulin during the period 55–70 min post injection (Table 2). However, the fractional clearance of platinum relative to inulin fell from 2.94 to 2.31 during this period. There are at least seven cisplatin metabolites present in the plasma at this time, each having a different value of the fractional clearance. The decline in the value for fractional clearance of total platinum during the 55–70 min period could therefore be due to the change in relative amounts of the plasma metabolites during this time. Moreover, it is apparent that fractional clearance measurements made after the administration of different doses of cisplatin, or made at different times after dosing, may lead to different results. This decline with time of the fractional clearance of platinum has been observed in humans treated with cisplatin [6] and may be responsible for the wide range of published values mentioned above.

Fractional clearance of cisplatin analogues

The renal tubular transport of platinum may be an important process in the development of the cisplatin induced renal lesion [3]. If this hypothesis were correct then it might have been possible to predict whether structural analogues of cisplatin would be nephrotoxic by measuring their fractional clearance. For this reason we measured the fractional clearance of non-nephrotoxic analogues of cisplatin, viz: *trans*-DDP, carboplatin and iproplatin. All of these compounds were found to have fractional clearance values not significantly different from cisplatin (Table 1). Siddik *et al.* [10] present data, from the rat, in which the renal and plasma clearances of carboplatin and inulin were similar; however, their protocol was not described. Harland *et al.* [11] report that in humans treated with carboplatin the ratio between the clearance of total free plasma platinum and the GFR averages 1.4 during the 24 hr following administration. Our data (Table 1) show no relationship between nephrotoxicity and fractional clearance, which suggests that the renal transport of platinum is not involved *per se* in nephrotoxicity, although in the case of cisplatin it may still be a prerequisite for nephrotoxicity.

Pharmacokinetic considerations

The *trans* isomer of cisplatin, *trans*-DDP, is more reactive than cisplatin [12] and more inhibitory to enzymes *in vitro* [13]; however, it does not cause nephrotoxicity *in vivo* (Table 5). The *trans* isomer binds very extensively to plasma proteins which leads to low platinum levels in pfp and as a result little platinum appears in the urine (Table 1). This pharmacokinetic phenomenon may prevent a large proportion of the dose reaching the kidney and may, in part, be responsible for the lack of nephrotoxicity of this compound *in vivo*.

The other analogues tested, carboplatin and iproplatin, are less reactive than cisplatin [14]. This is reflected in the smaller amounts of protein binding seen with these analogues compared to cisplatin (Table 1 and Fig. 1). The pfp levels of platinum from carboplatin are much higher than those from cisplatin 60–70 min after injection (Table 1). However, total plasma platinum and the urinary excretion of platinum are very similar for all treatments except for *trans*-DDP (Table 1).

Protein binding studies

Equilibrium dialysis (Fig. 1 and Table 3) shows that two types of protein binding occurs with these compounds. The binding of *trans*-DDP to protein is completely irreversible. With cisplatin the plasma proteins bind a small proportion of platinum reversibly, however, 65% remains firmly bound. Platinum from both carboplatin and iproplatin is largely reversibly bound (Table 3). The question therefore arises: Is this loosely attached platinum available for renal excretion? If this is the case then the correct fractional clearance value would be obtained by ignoring protein binding in the calculation of the

clearance of platinum. This would result in lower values for the fractional clearance of carboplatin (2.78) and iproplatin (2.04) compared to those shown in Table 1.

Modification of fractional clearance and nephrotoxicity of cisplatin by competitive inhibitors of renal tubular transport

The mechanisms involved in the renal tubular transport of platinum compounds are not known. Cisplatin is a neutral complex that appears to be transported unchanged. Some of its metabolites, which may be charged, are also transported [3]. Our results (Table 4) show that the mechanisms of renal transport of organic cations and anions, which are susceptible to competitive inhibition by other organic ions [18], do not currently provide a simple explanation for the renal transport of platinum species. Probenecid increases the fractional clearance of platinum (Table 4) and this is associated with an increase in the nephrotoxicity of cisplatin (Table 5), as has been reported previously [15]. Furosemide (200 mg/kg) causes a decrease in the fractional clearance of platinum (Table 4) but was without effect on nephrotoxicity (Table 5) at the dose studied (100 mg/kg). Furosemide decreases the concentration of platinum in the urine (Table 4) and the percentage dose excreted within 24 hr (Table 5). These results parallel those reported for the isolated rat kidney [5]. Ward *et al.* [16] have reported that furosemide can ameliorate cisplatin induced nephrotoxicity and this effect was ascribed to a reduction in the urinary platinum concentration. However, other workers [20] have shown furosemide to enhance cisplatin induced nephrotoxicity. The base triethanolamine (200 mg/kg) increased the fractional clearance of cisplatin, but did not affect nephrotoxicity (Table 5) at the dose studied (100 mg/kg). Although choline chloride (100 mg/kg) did not alter significantly the fractional clearance of platinum (Table 4), it markedly reduced nephrotoxicity (Table 5) at the lower dose (30 mg/kg) studied. Unfortunately choline chloride is toxic to rats [17] and this prevented higher doses being administered without deaths.

The observed fractional clearance of platinum is the nett result of the filtration, and differential reabsorption or secretion of at least seven platinum species [3, 5, 19]. If nephrotoxicity were brought about by a minor cisplatin metabolite it might be possible to inhibit the renal transport of the minor metabolite, and prevent nephrotoxicity, without a significant effect on the fractional clearance of total platinum. This may be the case when choline chloride is administered. Choline is bidirectionally transported in the kidney tubule [18], it therefore has the potential to block the reabsorption of a cationic species from the urine. The hydrolysis product of cisplatin, diaquodiammine platinum II, is a potential candidate for this role [8]. We aim to test the effect of choline on the renal transport of the individual cisplatin metabolites.

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REFERENCES

1. M. P. Hacker, E. B. Douple and I. H. Krakoff (Eds.), *Platinum Coordination Complexes in Cancer Chemotherapy*. Martinus Nijhoff, Boston (1984).
2. P. T. Daley-Yates and D. C. H. McBrien, in *Nephrotoxicity Assessment and Pathogenesis* (Eds. P. A. Bach, F. W. Bonner, J. W. Bridges and E. A. Lock), p. 356. Wiley, Chichester (1982).
3. P. T. Daley-Yates and D. C. H. McBrien, *Biochem. Pharmac.* **32**, 181 (1983).
4. P. T. Daley-Yates and D. C. H. McBrien, in *Platinum Coordination Complexes in Cancer Chemotherapy* (Eds. M. P. Hacker, E. B. Douple and I. H. Krakoff), p. 205. Martinus Nijhoff, Boston (1984).
5. P. T. Daley-Yates and D. C. H. McBrien, *Biochem. Pharmac.* **31**, 2243 (1982).
6. C. Jacobs, S. M. Kalman, M. Tretton and M. W. Weiner, *Cancer Treat. Rep.* **64**, 1223 (1980).
7. A. Heyrovsky, *Clin. Chim. Acta.* **1**, 470 (1956).
8. P. T. Daley-Yates and D. C. H. McBrien, *Biochem. Pharmac.* **33**, 3063 (1984).
9. N. M. Osman and C. L. Litterst, *Cancer Lett.* **19**, 107 (1983).
10. Z. H. Siddik, D. R. Newell, F. E. Boxall, M. Jones, K. G. McGhee and K. R. Harrap, in *Platinum Coordination Complexes in Cancer Chemotherapy* (Eds. M. P. Hacker, E. B. Douple and I. H. Krakoff), p. 90. Martinus Nijhoff, Boston (1984).
11. S. J. Harland, D. R. Newell, Z. H. Siddik, R. Chadwick, A. H. Calvert and K. R. Harrap, *Cancer Res.* **44**, 1693 (1984).
12. F. Basolo and R. G. Pearson, *Mechanisms of Inorganic Reactions*, Wiley, New York (1967).
13. P. T. Daley-Yates and D. C. H. McBrien, *J. appl. Toxic.* **1**, xi (1981).
14. M. J. Cleare, P. C. Hydes, D. R. Hepburn and B. W. Malerbi, in *Cisplatin Current Status and New Developments* (Eds. A. W. Prestayko, S. T. Crooke and S. K. Carter), p. 149. Academic Press, New York (1980).
15. P. T. Daley-Yates and D. C. H. McBrien, *Cancer Treat. Rep.* **68**, 445 (1984).
16. J. M. Ward, A. F. LeRoy and D. M. Young, *Cancer Treat. Rep.* **61**, 375 (1977).
17. P. G. Stecher (Ed.), *The Merck Index*. Merck, Rahway (1968).
18. B. R. Rennick, in *Methods in Renal Pharmacology, Vol. 4A, Renal Pharmacology* (Ed. M. Martinez-Maldonado), p. 335. Plenum Press, New York (1976).
19. P. T. Daley-Yates and D. C. H. McBrien, in *Platinum Coordination Complexes and Cancer Chemotherapy* (Eds. M. P. Hacker, E. B. Douple and I. H. Krakoff), p. 143. Martinus Nijhoff, Boston (1984).
20. W. Scholz, H. Grotsch, M. Hropot, H. Kief and E. Klaus, *Human Toxicol.* **3**, 447 (1984).