CHAPTER 9

Toxicokinetics of Ochratoxin A in Vervet Monkeys (*Cercopithecus Aethiops*)

The threat that OTA contaminated foods and feeds holds for both humans and animals were highlighted in **Chapters 2** and **3**. At first, the aim of this project was to develop methods for the analysis of OTA in biological samples using HPLC and LC/MS techniques and secondly to apply these methods to investigate the metabolism and toxicokinetics of OTA in vervet monkeys. This would lead to the calculations of the approximate half-life of OTA in humans and provide some insight in the role of OTA in putative human kidney diseases. The reasoning behind the use of vervet monkeys in these experiments was the supposition that the metabolism of monkeys is believed to be more similar to humans. The project was done in conjunction with the PROMEC group at the Medical Research Council, Tygerberg, Dr. G. Shephard, Ms. T.W. Nieuwoudt, Dr. J. Seier and Dr. V. Sewram; and Prof. E.E. Creppy, University of Bordeaux II, France. The development of the techniques applied in **Chapter 9** is summarised in **Chapter 10**. This chapter was recently submitted to *Journal of Toxicology and Applied Pharmacology* for publication and part of the work will also be communicated in a paper at the IUPAC Symposium on Mycotoxins and Phycotoxins in Brazil on May 2000.

Contribution made by the candidate

The candidate played the major role in the research and planning of the experiments, the cleanup of all the samples, the HPLC analyses of the urine-samples and compilation of the data. The author also assisted Ms. T.W. Nieuwoudt with the LC/MS analyses of the plasma samples. The administering of the toxin and the handling of the animals were done by the PROMEC group.

Toxicokinetics of ochratoxin A in vervet monkeys *(Cercopithecus aethiops)*

ABSTRACT

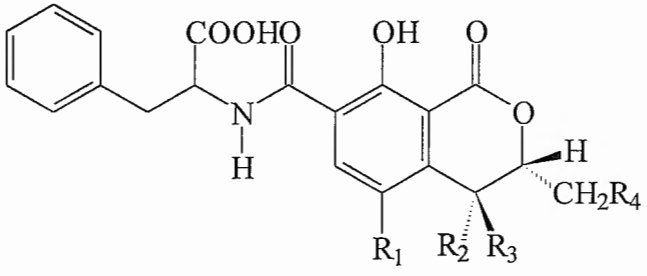
The toxicokinetics of ochratoxin A was investigated in vervet monkeys (*Cercopithecus aethiops)*. Three female monkeys were intravenously administered with 0.8 mg, 1.5 mg and 2 mg ochratoxin A per kg body weight (BW). Blood and urine were collected over a period of 21 days. Kidney function was monitored by measuring the chemical pathology parameters of the plasma. Plasma and urine extracts were analysed by liquid chromatography coupled to negative ion electrospray ionisation mass spectrometry and reversed phase high performance liquid chromatography equipped with fluorescence detection. The elimination half-life of ochratoxin A in the monkeys was determined to be 19-21 days and the average total body clearance was 0.22 ± 0.7 ml/h.kg and, the average apparent distribution volume of the central compartment 59 ± 9 ml/kg and of the peripheral compartment was 59 ± 20 ml/kg.

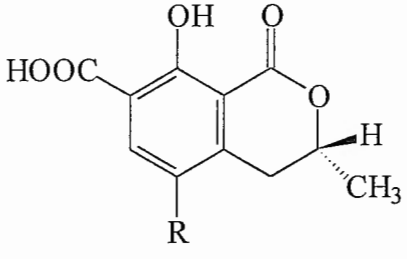
Keywords: Ochratoxin A, nephrotoxin, toxicokinetics, *Cercopithecus aethiops,* intravenously, negative ion electrospray ionisation mass spectrometry

INTRODUCTION

Ochratoxin A (OTA) an important nephrotoxin, teratogen and carcinogen, is mainly produced as a secondary metabolite of *Aspergillus aluceus* (formerly known as *A. ochraceus*) and *Penicillium verrucosum*. It contains a 5-chloro-3,4-dihydro-3-R-methyl–isocoumarin moiety linked to L-β-phenylalanine (Phe) through a 7-carboxy group (van der Merwe *et al.*, 1965, Bredenkamp *et al.*, 1989). The mycotoxin is a common contaminant of various foodstuffs including grains, coffee and wine (Zimmerli and Dick 1996). OTA accumulates in the kidney, liver and blood of animals. It is implicated in the aetiology of Balkan endemic nephropathy (BEN) and is the cause of Danish porcine nephropathy (Krogh, 1974, Szczech *et al.*, 1973, Stoev *et al.*, 1998). OTA has been found in human blood in a number of countries including Germany, Poland, Sweden, France and in high levels in the regions with BEN (Hald, 1991 and references cited, Creppy *et al.*, 1991, Maaroufi *et al.*, 1999). At molecular level it acts by competition with phenylalanine in the reactions catalysed by phenylalanine-tRNA synthetase and phenylalanine-hydroxylase and in addition it enhances the lipid

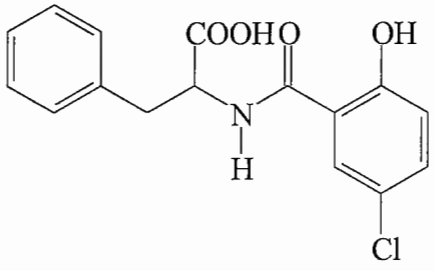
peroxidation process (Creppy *et al.*, 1984; 1990; Omar *et al.*, 1991). Toxicokinetic studies have been undertaken in various animals including rats, pigs, rabbits and chickens (Li *et al.*, 1997; Galtier *et al.*, 1979; Hagelberg *et al.*, 1989). The results of these studies have shown a two-compartment model with variation in half-lives among different animal species. OTA is cleaved to the essentially non-toxic ochratoxin α (OTα) in various organs of rats (Suzuki *et al.*, 1977; Hansen *et al.*, 1982; Stφrmer *et al.*, 1983). In the presence of nicotinamide adenine dinucleotide phosphate hydride (NADPH), pig and human liver microsomes incubated with OTA form both (4*R*)-hydroxyochratoxin A and (4*S*)-hydroxyochratoxin A, whereas rabbit liver microsomes also form, in addition to these two compounds, 10-hydroxyochratoxin A (see **Figure 1**). These oxidation reactions involve cytochrome P450 isoenzymes (Stφrmer *et al.*, 1981, 1983). Both these two epimers were also found in rat and rabbit liver (Stφrmer *et al.*, 1981) and in rat kidney (Stein *et al.*, 1985). OTA, ochratoxin α and (4*R*)-4-hydroxyochratoxin A are excreted in the urine of rats given OTA intraperitoneally or *per os* (Stφren *et al.*, 1982 a,b)





Ochratoxin A: R_1 = Cl, R_2, R_3 = H, R_4 = H
Ochratoxin B: R_1 = H, R_2, R_3 = H, R_4 = H
(4-*R*)-Hydroxyochratoxin A: R_1 = Cl, R_2 = OH, R_3 = H, R_4 = H
(4-*S*)-Hydroxyochratoxin A: R_1 = Cl, R_2 = H, R_3 = OH, R_4 = H
10-Hydroxyochratoxin A: R_1 = Cl, R_2 = H, R_3 = H, R_4 = OH

Ochratoxin α: R = Cl



N-(5-chloro-2-hydroxybenzoyl)-phenylalanine (internal standard)

Figure 1: Structures of the ochratoxins and the internal standard

The present experiments were performed by intravenous injection of OTA into vervet monkeys (*Cercopithecus aethiops)* at levels of 0.8; 1.5; 2 mg OTA/kg body weight (BW). The experiments were aimed at determining the half-life of OTA in vervet monkeys, monitoring the urinary excretion of OTA and detecting any sign of renal injury. The reasons for the choice of monkeys as experimental animals were firstly the presumption that metabolically these monkeys are more likely to resemble man than other animals and secondly owing to their size, they have the advantage that blood samples can be collected during the study without it being necessary to sacrifice the animals (Paine and Mars, 1999).

MATERIAL AND METHODS

Ochratoxin standards and chemicals

OTA was isolated from wheat, inoculated with *Aspergillus aluceus* (MRC 10582, CSIR, Pretoria) using the method of Stander *et al.*, (1999) (recrystallised from benzene, m.p. 91 °C, literature 90 °C, van der Merwe *et al.*, 1965). OTα was prepared by hydrolysing OTA in excess 6 M hydrochloric acid under reflux for 60 hours. (4*R*)-4-hydroxyochratoxin A, (4*S*)-4-hydroxyochratoxin A and 10-hydroxyochratoxin A were supplied by Prof. R. Marquardt, Department of Animal Science, University of Manitoba, Canada. N-(5-chloro-2-hydroxybenzoyl)-phenylalanine was synthesised according to Steyn and Payne, (1999). HPLC grade methanol and formic acid (Merck, Darmstadt, Germany) were used for LC-MS analysis. All other chemicals were of analytical grade.

Animals

The three vervet monkeys (*Cercopithecus aethiops*) used in the experiment were healthy adult females, 30-36 months old, weighing between 2.1 and 2.6 kg and bred in the Primate Unit of the Medical Research Council, Tygerberg, South Africa. They were housed separately in metabolic cages with free access to food and water. The monkeys were not sacrificed at the end of the experiments, but were returned to the breeding colony. The experimental protocol was approved by the Ethics Committee for Research on Animals of the Medical Research Council, Tygerberg, South Africa.

Dosage of OTA and collection of urine and blood

OTA was dissolved in 1 M sodium bicarbonate (1mg/ml) and administered intravenously to the monkeys at three different levels (0.8 mg/kg BW; 1.5 mg/kg BW and 2 mg/kg BW).
Blood samples (1.5-2 ml) for determination of OTA were drawn by venipuncture into tubes containing tripotassium ethylenediaminetetra-acetic acid (EDTA) as anticoagulant. Plasma was obtained by centrifugation at 1200 x g for 10 minutes at 4 °C and stored at –20 °C until it was analysed. For the determination of electrolytes (Na^+, K^+, Cl^-), creatinine and urea, separate blood samples were drawn and allowed to clot. Serum was obtained by centrifugation as above. Blood was drawn after 0, 0.5, 2, 4, 8, 24, 48 and 72 hours. Thereafter, blood samples were collected three times weekly. Samples for chemical pathology were drawn after 24, 48 and 72 hours and thereafter once weekly.
Urine samples were collected over the 24 hour period immediately prior to the drawing of plasma samples and stored at –20 °C until analysed.

Extraction of plasma

A salt solution was prepared by dissolving sodium chloride (5 g), 10 M hydrochloric acid (4 ml) and magnesium chloride (6.09 g) in distilled water to a total volume of 500 ml. Plasma (500 µl), salt solution (20 ml) and ethyl acetate (8 ml) were mixed, and subsequently put on ice (10 min) and centrifuged (3500 x g for 20 min). The ethyl acetate layer was removed (5 ml) and another portion of ethyl acetate (6 ml) was added to the water layer. After mixing, it was centrifuged (3500 x g for 20 min). The ethyl acetate layer (7 ml) was removed and the two ethyl acetate fractions were combined. The combined organic layers were washed with water (4 ml), and the ethyl acetate layer (10 ml) evaporated to dryness under a stream of nitrogen (60°C). An internal standard was prepared by dissolving N-(5-chloro-2-hydroxy benzoyl)-phenylalanine in methanol (5 µg/ml). The dried plasma extracts were reconstituted in the internal standard (350 µl) solution, sonicated (30 min), filtered through a syringe filter (0.45 µm, Millipore, Yonezawa, Japan) and injected (20 µl) onto the liquid chromatograph-mass spectrometer (LC-MS).

Extraction of urine

Each sample was divided into 3 portions – one to be extracted directly, one to be extracted after hydrolysis for 2 hours with glucuronidase and sulphatase (10 IU) from *Helix pomatia* (at 37°C and pH 5) and the third to be extracted after hydrolysis for 2 hours with (4 IU) of β-glucosidase (at 37°C and pH 5.5). The pH of these enzyme reactions was adjusted with 1 M HCl.

An EDTA solution was prepared by dissolving EDTA (0.2 g), sodium chloride (30 g) and *ortho*-phosphoric acid (1.5 ml) in distilled water to a volume of 100 ml. Urine (2 ml), EDTA solution (5 ml) and ethyl acetate (8 ml) were mixed well and centrifuged (3500 x g for 15 min). The ethyl acetate layer (5 ml) was removed and the water layer was re-extracted with ethyl acetate (6 ml). The combined ethyl acetate fractions were transferred into a vial and washed with distilled water (1.5 ml), centrifuged (3500 x g for 15 min). The ethyl acetate layer (7 ml) was then evaporated to dryness under a stream of nitrogen (60 °C). The urine extract was dissolved in methanol/water (300 µl, 1:1) sonicated (30 min) and 50 µl of the solution injected onto the HPLC.

Instrumentation

HPLC analysis was performed on a Spectra Series P2000 pump equipped with an AS 1000 autosampler and a UV 1000 variable wavelength UV detector (Thermo Separation Products Inc, Riviera Beach, FL, USA). Separation was achieved on a 150 x 4.6 mm Phenomenex Luna C_{18} reversed-phase column, with 5 µm particle size packing material (Phenomenex, Torrance, CA, USA), employing a flow rate of 0.5 ml/min and an isocratic mobile phase consisting of water/methanol/formic acid (30:70:0.1). Online UV detection was performed at 332 nm prior to MS detection. Quantification was performed by comparison of peak areas using an internal standard and Navigator software supplied by Finnigan MAT. Negative ion electrospray ionisation (ESI) mass spectrometry was performed using a Finnigan MAT LCQ ion trap mass spectrometer (San Jose, CA, USA). The MS parameters were optimised separately for the internal standard and OTA by direct infusion of the respective compounds into the ESI source. During LC-MS analysis, the LC eluent entered the mass spectrometer without splitting at a spray voltage of 5 kV for both OTA and the internal standard. The temperature of the heated capillary was maintained at 220 °C, the sheath gas flow was 60 arbitrary units, the auxiliary gas 10 arbitrary units, while the capillary voltage was set to -4 V.

The compounds of interest were detected using single ion monitoring (SIM), scanning for the respective deprotonated molecular ions within a narrow window of 10 mass units.

Three segments were employed during the chromatographic run; during segment one (0-15 minutes) OTα and the hydroxylated OTA species were monitored by scanning at *m/z* 250-260 and *m/z* 414-424, respectively, in segment two (15-19 minutes) the internal standard was monitored by scanning at *m/z* 317-327 and in the final segment (19-28 minutes) OTA was monitored by scanning at *m/z* 400-410 (see **Figure 2**).

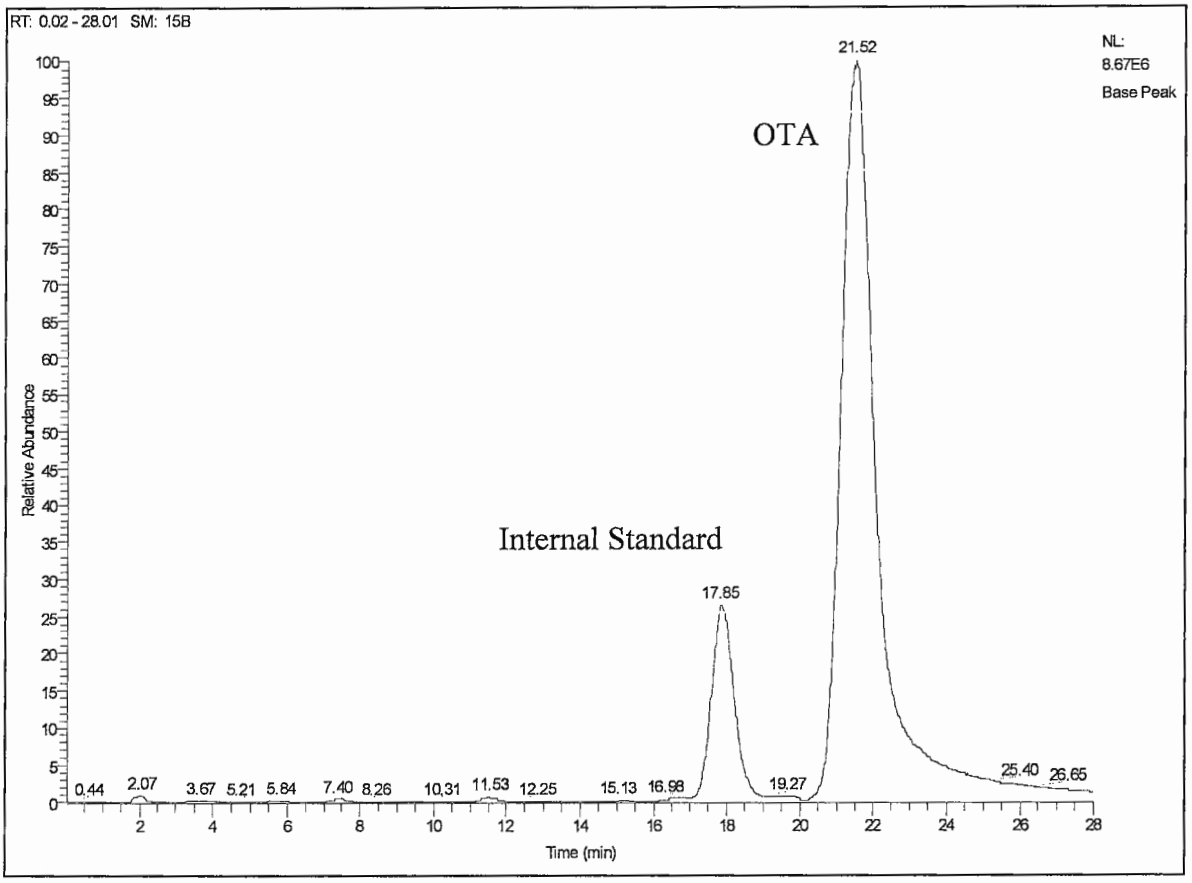


Figure 2: Single ion chromatogram of plasma extract from monkey 3, 30 minutes after dosing the monkey with OTA

Instrumentation for HPLC analyses of the urine

A Hewlett Packard 1090, HPLC system, fitted with a diode array (HP 1090) and fluorescence detector (HP 1100), autosampler and ChemStation software was used (Hewlett Packard, Waldbronn, Germany). Separations were achieved using a 4.6 mm × 150 mm, 5 μm, C_{18} reversed-phase column (Discovery, Supelco, Bellefonte, PA, USA) fitted with a C_{18} guard cartridge (Spherisorb ODS-2, Supelco) and a mobile phase of water/methanol/acetic acid

(50:60:2). The injection volume was 50 µl and a flow rate of 1 ml/min was used. The fluorescence detector was set at an excitation wavelength of 250 nm and an emission wavelength of 454 nm (see **Figure 3**).

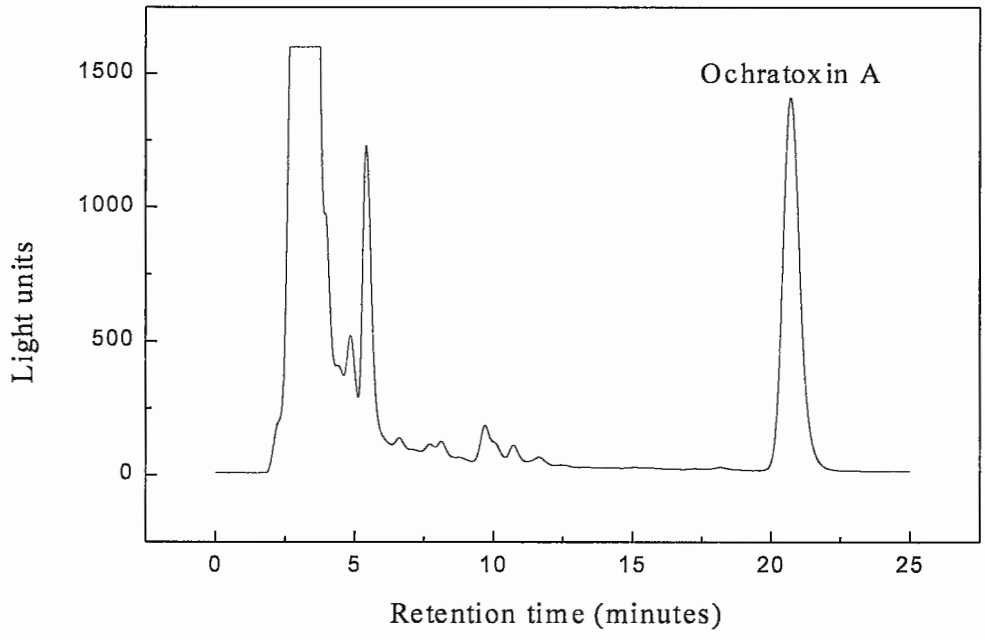


Figure 3: HPLC chromatogram with fluorescence detection of an extract of the urine of monkey 1, 5 days after the administration of OTA

Analysis of extracts

N-(5-chloro-2-hydroxybenzoyl)-phenylalanine was used as internal standard (IS) to compensate for the variation in the response of the LC-MS and resulted in highly reproducible peak areas [relative standard deviation (RSD) 1.3 % with n = 8, 5 ng OTA injection, calculated from the ratio of OTA and IS areas of an OTA standard]. The IS is a very stable structure-analogue of OTA, therefore, the parameters for the optimal detection of the two compounds were very similar. The LC-MS response was standardised daily and shown to be linear over the range employed for these analyses (10 to 250 ng of injected OTA). The linear calibration equations varied between y = 0.0074x and y = 0.0069x with correlation coefficients of 0.998 or better. The different ochratoxins, OTα (*ca.* 6 minutes), (4*S*)-4-hydroxyochratoxin A (*ca.* 8 minutes) and (4*R*)-4-hydroxyochratoxin A (*ca.* 9 minutes), OTA (*ca.* 21.5 minutes) and the internal standard (*ca.* 18 minutes) separated effectively under the HPLC conditions employed. All extractions and injections onto the LC-MS were done in

duplicate, yielding a total of 4 analyses per sample. The limit of quantitation was an injection of 2 ng OTA with a signal to noise ratio (S/N) of 10 and the limit of detection was an injection of 0.5 ng OTA (S/N ≥3).

The recovery of the OTA standard added to monkey plasma was 92.6 ± 3.0% [mean ± RSD, n=4] at a 5 ng OTA injection.

The detection limit for OTA in urine measured by HPLC with fluorescence detection was 0.5 ng (S/N ≥ 3) and the limit of quantitation 10 ng (S/N = 10). The average recovery was 84± 6% [mean ± RSD, n=4] at a 1.5 μg/ml level. The analysis of the urine and plasma samples showed no detectable levels of OTα and of the hydroxylated OTA derivatives [HPLC, LC-MS and silica gel TLC (toluene/acetic acid, 4:1) evidence].

RESULTS

No adverse effects were noted in any of the monkeys over a period of 21 days after OTA was administered, and the monkeys appeared healthy and showed no evidence of reduced feed consumption. Urea, creatinine and electrolyte levels stayed within the normal boundaries throughout the experiment (**Table 1**).

Figure 4 shows levels of OTA in the plasma of the three monkeys after different time periods. Only OTA, and none of its known metabolites, was detected in the plasma, with a maximum measured value (C_{max}) at 2 hours after administering the toxin.

HPLC and LC-MS analysis of the urine of the monkeys failed to detect any hydrolysis products. The data of the subsequent clearance of the toxin are presented as a plot of the natural logarithm of concentration of OTA against time (**Figures 5-7**), a perusal of the data verified the similar toxicokinetic trends of the OTA in the three monkeys. No OTA was detected in the plasma and urine of the monkeys prior to the administration of OTA.

Table 1: Results of the biochemical pathology of the serum of the monkeys.

Monkey	Day	Na^+	K^+	Cl^-	Urea	Creatinine
1	0	147	3.7	107	1.8	50
1	1	149	4.4	113	5.3	49
1	2	155	3.9	111	2.8	51
1	5	152	3.8	115	4.5	58
1	14	150	3.6	113	3.3	55
1	21	150	3.8	107	1.8	61
2	0	145	3.9	111	2.5	57
2	1	145	3.5	112	8.8	69
2	2	152	3.6	114	5.4	78
2	5	142	2.9	106	6.4	100
2	14	144	2.8	110	2.3	111
2	21	145	3.5	103	1.7	87
3	0	142	3.8	109	2.1	62
3	1	145	3.5	108	6.9	79
3	2	152	3.9	107	5.2	92
3	5	146	3.2	109	6.8	116
3	14	148	3.7	114	2.6	125
3	21	148	3.7	110	1.7	102

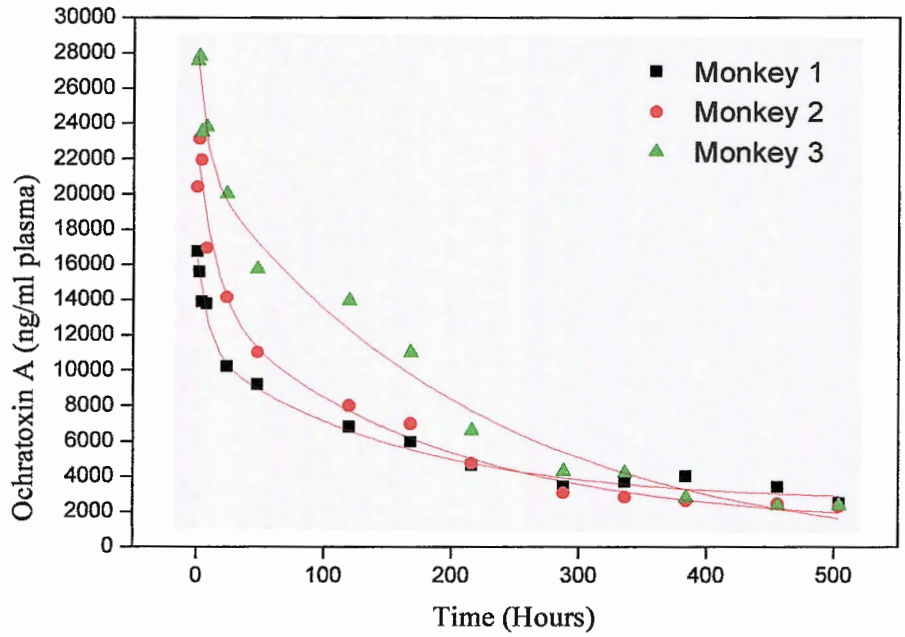


Figure 4: OTA levels in the plasma of the respective monkeys at different time periods following the administration of the toxin to the monkeys

The clearance of OTA from the plasma suggested a two-compartment model with the following bi-exponential equation (Shargal and Yu, 1985):

$$Cp_{iv} = Ae^{-\alpha t} + Be^{-\beta t}$$

Cp_{iv} is the plasma concentration at any given time t, A and B are the intercepts obtained from the logarithmic plot of Cp_{iv} versus t and α and β are the rate constants of the two exponential components of the curve (See **Table 2**). This model comprises two compartments to which a drug is distributed (Shargal and Yu, 1985).

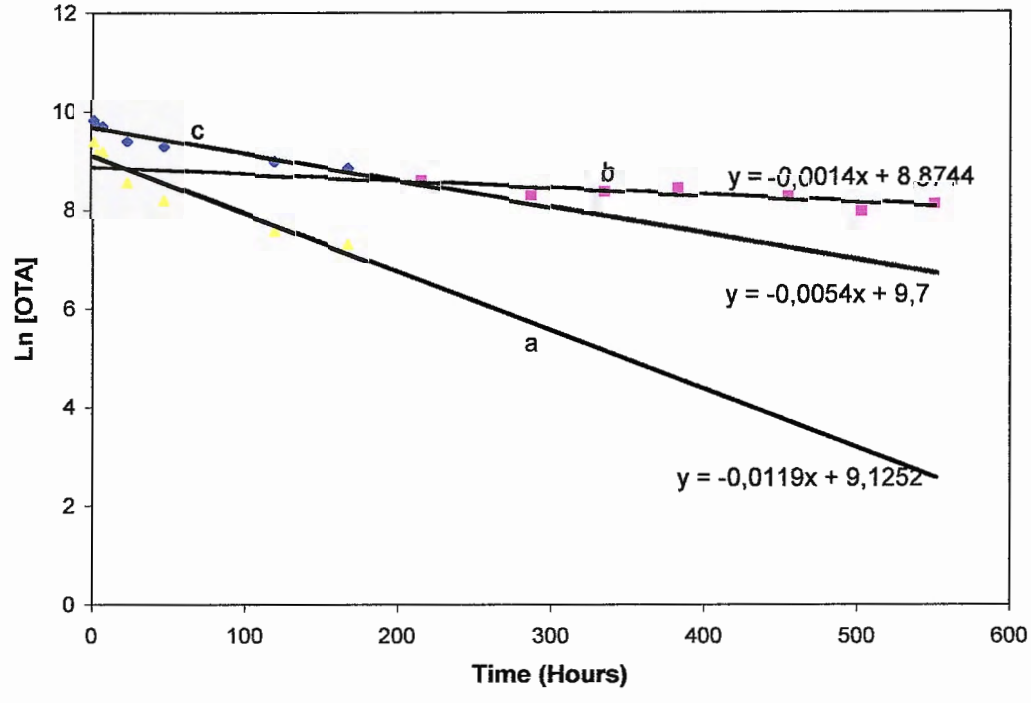


Figure 5: Graph of the natural logarithm of the OTA concentration in the plasma (ng/ml) of monkey 1 (lines c and b) and the calculated C_p-C'_p values (line a) versus time

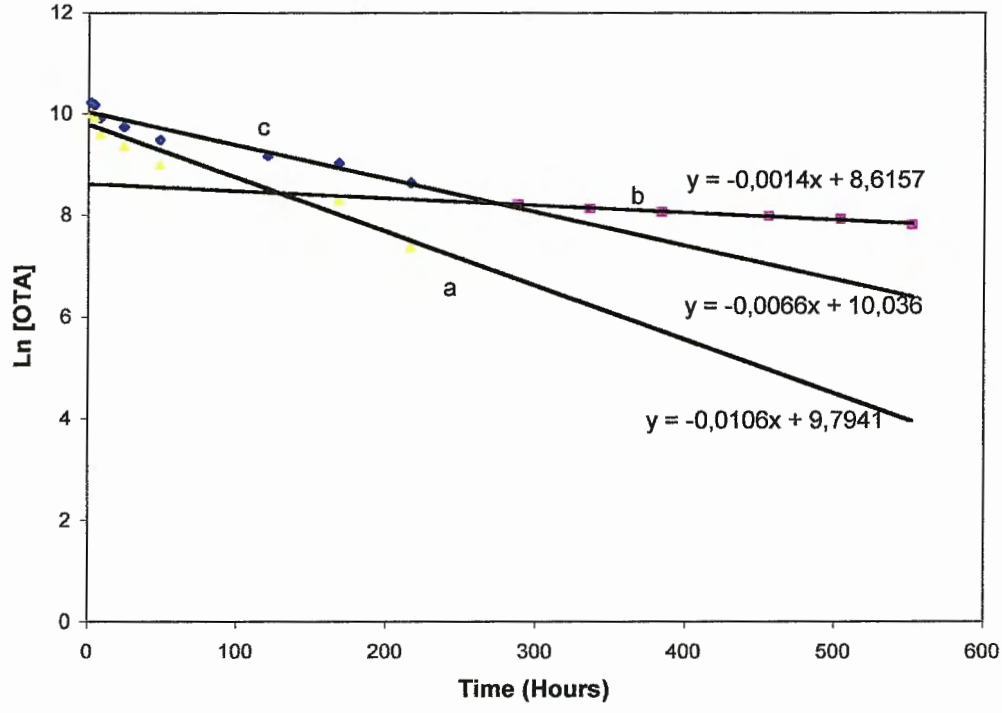


Figure 6: Graph of the natural logarithm of the OTA concentration in the plasma (ng/ml) of monkey 2 versus time (lines c and b) and the calculated C_p-C'_p values (line a)

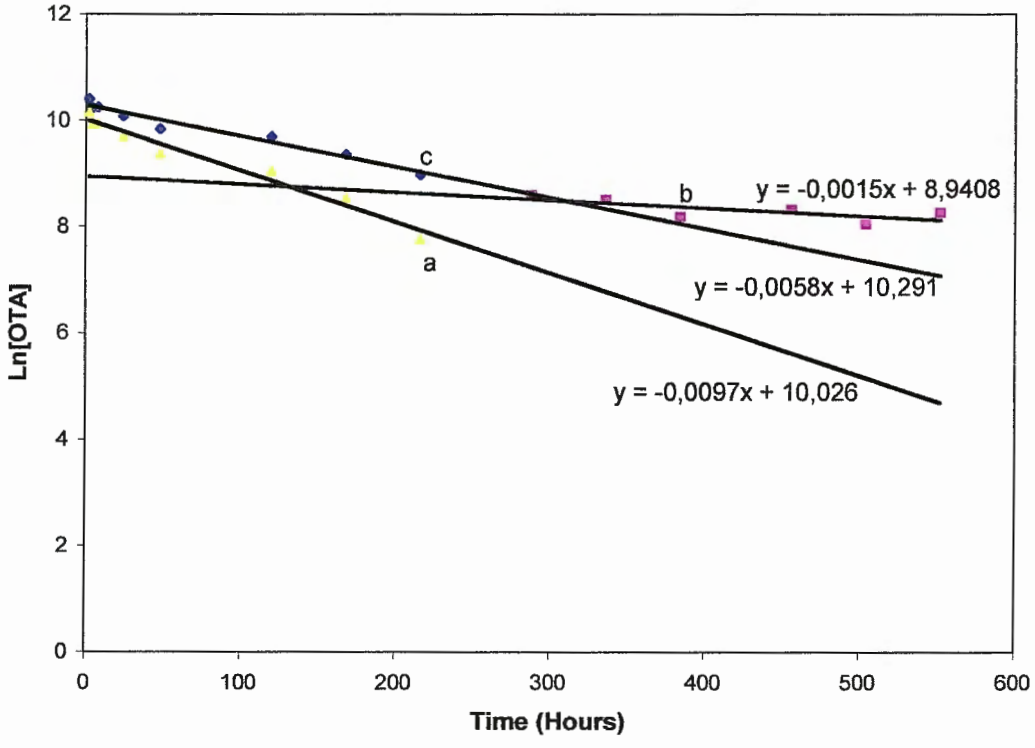


Figure 7: Graph of the natural logarithm of the OTA concentration in the plasma (ng/ml) of monkey 3 versus time (lines c and b) and the calculated C_p-C'_p values (line a)

The experimental data were fitted to a curve by the method of residuals. It is evident from the curve (**Figure 4**) that the initial distribution rate is quicker than the elimination rate, yielding α to be larger than β. The $t_{1/2}$ of the elimination phase was calculated from:

$$t_{1/2} = 0.693/\beta$$

By subtracting the experimental data, c from the values of the extrapolated line b, a new line, a, was constructed with the natural logarithm of the residual plasma concentration (Cp-Cp') which represents the α (distribution) phase.

The A, α and $t_{1/2}\alpha$ values were then calculated in a similar way as B, β and $t_{1/2}\beta$ (Shargal and Yu, 1985).

Table 2: Dosage of OTA plasma half-lives, C_{max}, weights and calculated toxicokinetic parameters of the three monkeys

	Monkey 1	Monkey 2	Monkey 3
OTA Dosage	0.8 mg/kg BW	1.5 mg/kg BW	2.0 mg/kg BW
$t_{1/2}\alpha$	58.2 hrs	65.4 hrs	71.8 hrs
$t_{1/2}\beta$	495 hrs	495 hrs	462 hrs
α	0.0119 hr^{-1}	0.0106 hr^{-1}	0.0097 hr^{-1}
β	0.0014 hr^{-1}	0.0014 hr^{-1}	0.0015 hr^{-1}
A	9184 ng/ml	17928 ng/ml	22539 ng/ml
B	7147 ng/ml	5518 ng/ml	7637 ng/ml
CL_T	0.14 ml/h.kg	0.26 ml/h.kg	0.27 ml/h.kg
$C_U \times V_U$	55979 ng	50991ng	54199 ng
C_{mid}	12275 ng/ml	16982 ng/ml	23995 ng/ml
CL_R for the first 48 hours	0.095 ml/h	0.063 ml/h	0.047 ml/h
Dose	1.72 mg	3.30 mg	5.58 mg
k_{21}	0.0060	0.0036	0.0036
k_{10}	0.0028	0.0042	0.0041
k_{12}	0.0045	0.0043	0.0036
Vc	48.99 ml/kg	62.28 ml/kg	66.04 ml/kg
V_p	36.98 ml/kg	74.63 ml/kg	65.67 ml/kg
OTA in plasma after 2 hrs	18.3 µg/ml	27.8 µg/ml	33.4 µg/ml
Weight	2.15 kg	2.26 kg	2.80 kg

The total plasma clearance (CL_T) is the volume of plasma cleared of compound per unit time and represents the sum of all the individual clearance processes such as metabolism (CL_M), renal excretion (CL_R) and biliary excretion (CL_B).

The renal clearance for the first 48 hours was calculated as follows:

$$CL_R = (C_U \times V_U)/(\Delta t \times C_{mid})$$

Where C_{mid} is the concentration in plasma at the mid-point of urine collection and $C_U \times V_U$ is the total amount excreted in the urine over a time Δt (48 hours).

The volume of distribution of the central compartment (Vc), the apparent volume of the peripheral compartment (V_p), the rate of elimination (k_{10}), and the distribution rate constants (k_{12} and k_{21}) were calculated as follows (Li *et al.*, 1997):

$$Vc = dose/[(A+B) \times BW]$$

$$k_{21} = (A\beta + B\alpha)/(A+B)$$

$$k_{10} = \alpha\beta / k_{21}$$

$$k_{12} = \alpha + \beta - k_{21} - k_{10}$$

$$V_p = (V_c \times k_{12})/ k_{21}$$

$$CL_T = k_{10} \times V_C$$

The value of any parameter (P) can be deduced by using allometry, a mathematical extrapolation based on the body weight of an animal (Calder, 1981), if the parameter is known in two or more species.

$$P = aW^x$$

The basis for this relation, is the assumption that the underlying similarities in mammalian species viz. the organs of absorption, distribution and elimination are similar (Renwick, 1999). Some of the toxicokinetic profiles of OTA as reported in the literature on a number of species are summarised in **Table 3**. The elimination half-life of OTA in a 70 kg human was calculated to be 46 days (2773 h) by using allometry and the elimination half-lives of OTA in the mammalian species in **Table 3** ($y = 0.52x + 0.92$ for the log ($t_{1/2}\beta$) *versus* log (BW) curve with $R^2 = 0.947$)[See **Figure 9**]. Although the toxicokinetic data of OTA in swine (pigs) are well documented, it was not used in the calculations because the half-life is much shorter relative to the body size should swine be compared to the other mammals.

Treatment of the urine samples with glucoronidase and sulphatase, and β-glucosidase yielded the same results as the control urine samples, indicating that there were no significant quantities of simple OTA-glucosides, -glucoronides or -sulphates excreted in the urine. The highest measured amount of OTA was excreted in the urine after 5 days following administration of the toxin to the three monkeys (See **Figure 8**).

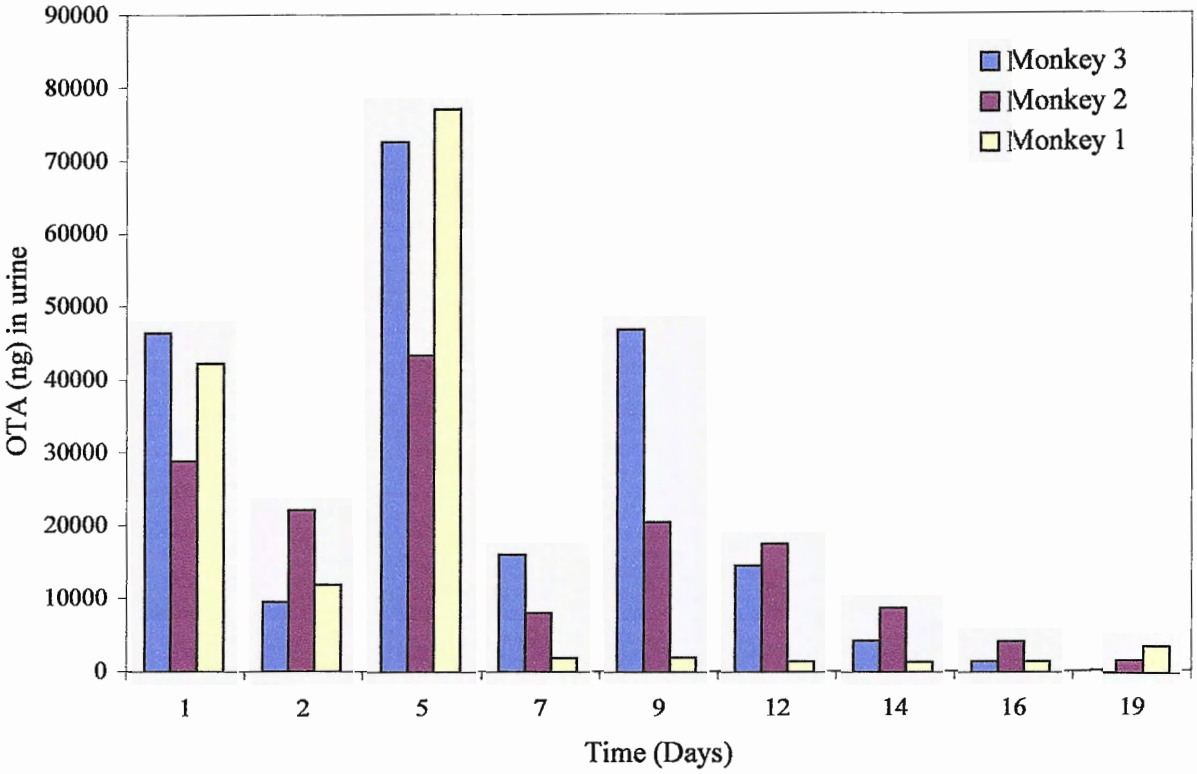


Figure 8: OTA excreted in the urine during the first 19 days following administration of OTA to the monkeys

DISCUSSION

In all species (mammals, fish and birds) studied to date, OTA has exhibited second order elimination kinetics characterized by a two compartment model (Table 3). Toxicokinetic studies have generally revealed slow plasma clearances and, consequently, long half-lives. These effects, may be attributed to the very high affinities of the toxin to plasma proteins in these species; association constants for OTA of the high-affinity macromolecules in e.g. human and pig plasma were found to be $2.3 \times 10^{10}\ M^{-1}$ and $0.6 \times 10^{10}\ M^{-1}$ respectively (See Marquardt and Frohlich, 1992 for a review). The calculated half-life of OTA in humans (46

days) by using the data of the more sensitive mammals to OTA is indicative to the possible health hazards that the regular intake of OTA contaminated foods hold for humans. Our data would clearly suggest that OTA consumed over extended periods of time might result in the accumulation of potentially hazardous toxins in the body. The changes in the urea, creatinine and electrolyte levels of all the monkeys throughout the experiment indicate that the administered OTA had no significant effects on the kidney function of the three monkeys (**Table 1**). The plasma half-life of other mycotoxins in vervet monkeys e.g. fumonisins (40 min) is much lower than that of OTA (Shephard *et al.*, 1994).

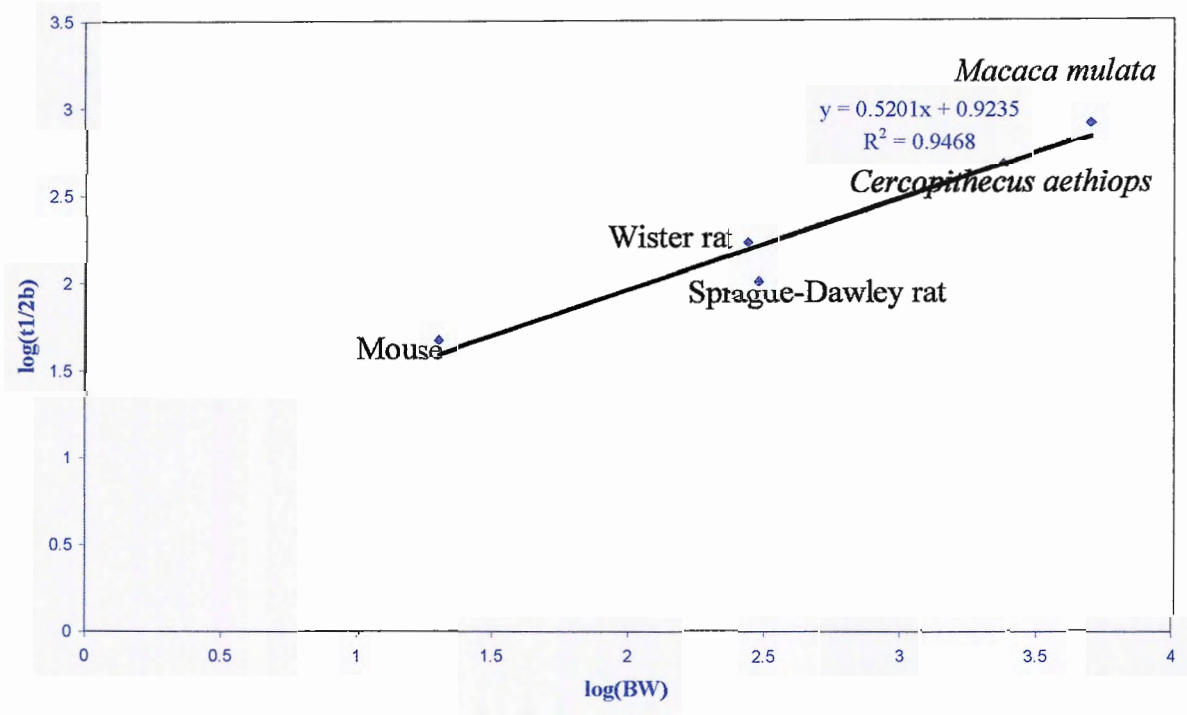


Figure 9: Inter-species scaling applied to the elimination half-life of OTA

In conclusion, this study was the first to determine the toxicokinetics of OTA in vervet monkeys. No evidence was found for any appreciable metabolic conversion of the toxin in the monkeys, viz. the absence of OTα or hydroxylated derivatives. The formation of hydroxylated OTA derivatives by oxidase systems in the liver, kidney and other tissues of rats as well as OTα by intestinal microorganisms are well documented (See Marquardt and Frohlich, 1992 for a review). The absence of OTα in the plasma/urine in the current study is due to the intravenous administration of the toxin: No OTA has been subjected to hydrolysis in the gastrointestinal tract.

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Table 3: Toxicokinetic profiles of OTA in a number of species after intravenous injection

Specie	Weight (g)	Dose	$t_{1/2}\beta$ (h)	V_d (ml/kg)	CL (mg/kg.h)	Reference
Monkey (*Macaca mulata*) male	5 000	50 ng/g	840	200	0.17	Hagelberg *et al.*, (1989)
Vervet monkey (*Cercopithecus aethiops)* female	2 400	0.8-2 mg/kg	484	118 ± 29	0.22 ± 0.7	
Fish (*Cyprinus carpio*)	1 000	50 ng/g	8.3	690	57	Hagelberg *et al.*, (1989)
Quail (*Coturnix coturnix japonica*)	160	50 ng/g	12	1500	66	Hagelberg *et al.*, (1989)
Mouse (NIH Bethesda) male	20	50 ng/g	48	420	8.1	Hagelberg *et al.*, (1989)
Rat (Wistar) male	250-300	50 ng/g	170	230	0.92	Hagelberg *et al.*, (1989)
Rats (Sprague-Dawley)	300	0.03 mg/kg	103±16		3.11	Li *et al.*, (1997)

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