1   **Toxicokinetics of fumonisin B1 in turkey poults and tissue persistence after**

2   **exposure to a diet containing the maximum European tolerance for fumonisins**

3   **in avian feeds**

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19   **Running title:** Toxicokinetic and persistence of fumonisin B1 in turkey poults
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1 **Abstract**

2 The kinetic of fumonisin B1 (FB1) after a single IV and oral dose, and FB1 persistence in tissue were

3 investigated in turkey poults by HPLC after purification of samples on columns. After IV administration

4 (single-dose: 10 mg FB1/kg bw), serum concentration–time curves were best described by a three-

5 compartment open model. Elimination half-life and mean residence time of FB1 were 85 and 52 min,

6 respectively. After oral administration (single-dose: 100 mg FB1/kg bw) bioavailability was 3.2%;

7 elimination half-life and mean residence time were 214 and 408 min, respectively. Clearance of FB1

8 was 7.6 and 7.5 ml/min/kg for IV and oral administration respectively. Twenty four hours after the

9 administration of FB1 by the intravenous route, liver and kidney contained the highest levels of FB1 in

10 tissues, level in muscle was low or below the limit of detection (LD, 13 µg/kg). The persistence of FB1

11 in tissue was also studied after administration for nine weeks of a feed that contained 5, 10 and 20 mg

12 FB1+FB2/kg diet. Eight hours after the last intake of 20 mg FB1+FB2/kg feed (maximum

13 recommended concentration of fumonisins established by the EU for avian feed), hepatic and renal

14 FB1 concentrations were 119 and 22 µg/kg, level in muscles was below the LD.

15

1 Introduction

2 Fumonisin B1 (FB1) is the major mycotoxin produced by *Fusarium verticillioides* and *Fusarium*

3 *proliferatum* fungi which are widely found in corn and corn screenings (EHC, 2000). This mycotoxin is

4 suspected of being involved in esophageal cancer in humans and was reported to be carcinogenic in

5 rodents (JECFA, 2001; IARC, 2002). In farm animals, equine leukoencephalomalacia and porcine

6 pulmonary edema are two syndromes known to be caused by FB1. Hepatic and renal toxicity can be

7 observed in several species, including lambs, rats, broilers, turkeys and ducks (EHC, 2000; Bailly et

8 al., 2001; Tran et al., 2005; Tardieu et al., 2007).

9 FB1 is a polar mycotoxin that is weakly absorbed and rapidly excreted in all species studied (JECFA,

10 2001). Only small amounts of FB1 were detected in serum and tissues after oral administration,

11 indicating that absorption is weak (less than 5% of the dose). FB1 was distributed to most tissues but

12 the liver and kidney retained most of the absorbed toxin (JECFA, 2001; Meyer et al., 2003; Fodor et

13 al., 2006). When FB1 was evaluated by the joint committee of the FAO and OMS, there was little or no

14 evidence that FB1 is metabolized in vitro or in vivo in animals, the material retained in liver and kidney

15 being primarily unmetabolized FB1 (EHC, 2000; JECFA, 2001). However, because little FB1 was

16 retained in tissues, it was concluded that fumonisin residues in food products derived from animals are

17 insufficient to make them hazardous to consumers (EHC, 2000; JECFA, 2001). Recent studies have

18 demonstrated that swine caecal microbiota can metabolize FB1 to partially hydrolyzed FB1 (PHFB1)

19 and to few amounts of aminopentol (AP) (Fodor et al., 2007). Studies in weaned piglets revealed the

20 presence of PHB1 and AP in tissue but confirmed that unmetabolized FB1 was the most abundant

21 (Fodor et al., 2008).

22 FB1 toxicokinetic has been widely studied using radiolabeled toxin. When [14C]FB1 is dosed by

23 intraperitoneal or intravenous injection, initial elimination is rapid with a half-life of approximately 10 to

24 60 min in rats, monkeys, cows and laying hens (Shephard et al., 1992b,c; Norred et al., 1993;

25 Vudathala et al., 1994; Shephard et al., 1994; Prelusky et al., 1995). The elimination kinetics based on

26 intraperitoneal or intravenous dosing is consistent with a one- or two-compartment model. Similar

27 results were obtained in rat by the use of unlabeled FB1 (Martinez-Larranaga et al., 1999). In pigs,

28 clearance of [14C]FB1 from blood following an intravenous injection was best described by a 3-

29 compartment model, the half-life of the terminal phase being around 182 min (Prelusky et al., 1994).

1   Because FB1 is poorly absorbed from the gastrointestinal tract and extensively distributed in tissues,

2   the elimination kinetics following oral dosing is not as easily described. The majority of 14C label

3   dosed orally was recovered in feces (approximately 90%) with less than 1% recovered in urine

4   (Norred et al., 1993; Prelusky et al., 1994). In pigs dosed intragastrically, half-life was determined to

5   be 96 min (Prelusky et al., 1994) whereas in laying hens, it was 116 min (Vudathala et al., 1994). In

6   pigs, it was estimated that a withdrawal period of at least 2 weeks would be required to eliminate the

7   toxin from the liver and kidneys (Prelusky et al., 1996). Additional studies in weaned piglets confirmed

8   that the absorption of FB1 is weak (3.9%) and that FB1 and to a less extend PHB1 and AP were

9   detected in tissue during a 10-day long elimination period (Fodor et al., 2008).

10   Although numerous data report the presence of fumonisins in corn and corn products (EHC, 2000;

11   JECFA, 2001), only few studies concerning the presence of the toxin in food products obtained from

12   animals were done. In France, a complete analysis of the contamination of food by FB1 revealed that

13   avian kidney and liver may contain more than 100 µg FB1/kg of tissue (Collectif INRA-DGAL 2004),

14   suggesting that human exposure to FB1 by the ingestion of food products derived from animals should

15   be considered.

16   The purpose of this study was to investigate the toxicokinetics of FB1 in turkeys after intravenous and

17   oral dosing. The elimination kinetics based on intravenous dosing is consistent with a two- or three-

18   compartment model. At the end of the study, the liver and kidney retained most of the absorbed

19   material. FB1 was also observed in these tissues after the administration of diet containing 5 to 20 mg

20   FB1+FB2/kg feed, the last concentration being the maximum European tolerance for fumonisins in

21   avian feeds (J.O. UE, 2006).

22

23   **Material and Methods**

24   All experimental procedures involving animals were in accordance with the French National Guidelines

25   for the care and use of animals for research purposes.

26

27   Production of Fumonisins

28   Fumonisins were produced using a highly toxinogenic strain of *F verticillioides* (NRRL-3428) isolated

29   from corn in the course of equine leucoencephalomalacia (Bailly et al., 2005). Briefly, autoclaved

30   maize was inoculated with approximately 10^6 spores (1 cm2 of 1-week subculture on PDA). Flasks

were incubated for 5 weeks at 20℃. Maize cultures were extracted by mechanical agitation overnight with acetonitrile/water (1v:1v). The crude extracts were filtered and concentrated by acetonitrile evaporation. The amount of fumonisins was determined by HPLC as described below. The average purity of the rough extract obtained was 54% FB1, 8% FB2 and 9% FB3. Twenty-nine percent of the extract comprised pigments of corn. The rough extract was then purified on SAX columns. Ten ml of the extract was filtered (Fiorini filter N 3) (VWR, Fontenay sous Bois, France) and applied to SAX columns (500mg, 2.8 ml) (VWR, France). Columns were eluted with 14 ml of acidified methanol (acid acetic 0.5%). The eluates were pooled and evaporated until dry under flows of nitrogen and analyzed by HPLC. The output of the purification process was 96 ± 2%. The purified extract was diluted in water to obtain a solution containing 10mg FB1/ml, which was directly administrated to turkeys during the kinetics studies.

Preparation of feed

The feed was formulated and manufactured at the experimental station of Boigneville (ARVALIS – *Institut du végétal*, Boigneville, France) according to usual practices in order to provide equal protein contents and energy intake, as well as to meet amino acid (lysine, sulfur amino acids, and tryptophan) and mineral requirements. A starter diet and two growth diets were prepared in the form of a mixture of raw materials not contaminated by mycotoxins and a mixture including different percentages (0 to 20%) of a batch of corn contaminated by fumonisins (FB1 + FB2 = 117 mg/kg) and of a batch of corn of the same origin that was not contaminated (12 to 32%). The levels of contamination of the final feed were 0, 5, 10 and 20 mg FB1 + FB2/kg. The absence of other mycotoxins was confirmed by chromatography and/or ELISA tests (concentrations of aflatoxin B1, ochratoxine A, zearalenone, deoxynivalenol and T2 toxin respectively lower than 10, 10, 50, 250 and 50 µg/kg).

Animals and samples

Sixty one-day-old male turkeys of the BUT 9 strain (Sicamen, Volnay, France) were placed in cages in groups of 2 at the experimental station of Pouline (ARVALIS – *Institut du végétal*, Pouline, Villerable, France) with free access to feed. The non-contaminated starter diet was distributed to all the turkeys for one week. At the end of this phase of adaptation, turkeys were placed in individual cages and fed *ad libitum* for nine weeks with the feeds prepared as described above. Weight and feed consumption

were measured weakly. Toxicokinetic studies were conducted on turkeys fed with the non-contaminated diet whereas persistence of FB1 in tissues was studied on turkeys fed the 5, 10 and 20 mg FB1+FB2/kg diets.

For the toxicokinetic studies, the animals had free access to feed for 15 minutes at the beginning of the luminous program, the animals were then weighed and a blood test was carried out, i.e. at time 0 of the kinetic study. For the study by the intravenous (iv) route, eight animals received 10 mg of FB1/kg body weight at a volume of 1 ml/kg. The animals were put back in their cages with free access to food. Blood samples (2 ml) were taken 3, 5, 10, 20, 30, 45, 60, 90, 120, 240, 360, 540 and 1200 minutes after administration of the toxin. For the study by the oral route, eight other animals received 100 mg of FB1/kg body weight at a volume of 10 ml/kg. The animals were put back in their cages with free access to food. Blood samples (2 ml) were taken 30, 60, 120, 180, 300, 420 and 600 minutes after administration of the toxin. All blood samples were removed from the jugular vein on dry tubes and centrifuged for 15 min at 3000 g after coagulation. The serum was removed and stored at −20℃ until use. All the animals were killed 24 and 10 hours after the administration of FB1 by the intravenous and the oral route respectively, weighed and a post-mortem examination was carried out to reveal a possible pathology. Ten grams of muscle, liver and kidney were taken and stored at -20C until analysis.

For the study of the persistence of FB1 in tissues, feed was removed eight hours before killing. Blood samples were collected before killing as previously described. The different organs were separated and weighed to measure the possible effect of fumonisins on tissue mass. Ten grams of muscle, liver and kidney were removed and stored at -20℃ until a nalysis.

Preparation of samples

All operations were performed at room temperature. Solid-phase anion-exchange column cleanup was used for the purification of plasma samples whereas immunoaffinity column cleanup was used for the purification of tissue samples according to Shephard et al. (1992a) and Visconti et al. (2001) modified as follow.

Five hundred µl of borate buffer (0.1 M, pH 5.8) and 750 µl of acetonitrile were added to 250 µl of serum. Samples were first placed on a stir table for 30 minutes at 300 rpm and centrifuged for 15 minutes at 3000 g. The supernatant was defatted on a C18 Supelclean column (3 ml, Suppelco, USA).

Elution was done by borate buffer (pH 5.8) and acetonitrile (3 ml each). The eluate was passed through a SAX cartridge (Bondelut, Varian, Harbor City, USA) and rinsed with 8 ml methanol/water (75/25, v/v)) and 3 ml methanol. FB1 was eluted by 10 ml of methanolic acetic acid (99/1 v/v). The eluate was evaporated at 40 ℃ in the dark under a gentle stream of nitrogen. Dry residue was suspended with 250 µl of acetonitrile/water (v/v), vortexed and ultrasonicated for 5 min. FB1 content was determined as described below.

Two ml of distilled water and 25 mg of NaCl were added to the tissue (1 gram) for a homogenization step performed with a Teflon Potter (500 rpm). The total homogenate was collected and 2 ml of acetonitrile/methanol (50/50, v/v) were added. Samples were placed on a stir table for 120 minutes at 300 rpm and centrifuged for 15 minutes at 3000 g. Three ml of the supernatant fraction were defatted twice with 4 ml of hexane and centrifuged for 15 minutes at 3000 g. Two ml of the aqueous phase were diluted with 8 ml of Phosphate Buffer Saline, pH 7.4 (PBS). As recommended by the manufacturer, this solution was passed through a FUMONIPREP cartridge (R. Biopharm Rhône LTD, Glasgow, Scotland). The column was washed with 10 ml of PBS, pH 7.4. FB1 was eluted with 1.5 ml of methanol and 1.5 ml of water. The eluate was evaporated at 40℃ in the dark under a gentle stream of nitrogen. Dry residue was suspended with 200 µl of acetonitrile/water (1:1), vortexed and ultrasonicated for 5 min. FB1 content was determined as described below.

FB1 derivatization and HPLC analysis

FB1 was derivatized to make a fluorescent derivative and to be able to quantify it with sufficient accuracy. The samples were derivatized with OPA solution (5 mg O-phtaldialdehyde, 2.5 ml acetonitrile, 5 µl beta-mercaptoethanol): briefly, 25 µl of OPA solution, 25 µl of borate buffer 0.1M pH 8.3, 25 µl of water were added to 25 µl of purified extract. After one minute of incubation, the derivatized mixture (20µl) was injected on the chromatographic system: a M 2200 pump (Bischoff, Leonberg, Germany) connected to a Prontosil C18 column (250 x 4.6 mm, Bischoff, Leonberg, Germany). Fluorescence was detected by a RF 10A XL fluorimeter (Shimadzu, Japan). The chromatograms obtained were monitored by PIC 3 software (ICS, Toulouse, France).

The mobile phase was composed of methanol/phosphate buffer (NaH2PO4 0.1 M pH 3.35, 75/25, v/v). The flow rate was 1 ml/min. The excitation and emission wavelengths were respectively 335 and

1   440 nm. The retention time of FB1 was around 10 min. FB1 was quantified by measuring the peak

2   area and comparing it to a standard calibration curve. The mean of recovery was 60%.

3

4   Data analysis

5   Serum concentrations of FB1 after IV and oral dosing were plotted against time. The curves were

6   fitted by non-linear least-squares analysis with the SigmaPlot Software (Systat software Inc.,

7   http://www.systat.com/products/sigmaplot/). The serum curve of FB1 obtained for each animal after a

8   single IV dose was fitted using the two- and the three-compartment models according to the following

9   exponential equations:

10     $f=A*exp(-\alpha*x)+B*exp(-\beta*x)$ (two exponentials model)

11     $f= A*exp(-\alpha*x)+ D*exp(-\delta*x)+B*exp(-\beta*x)$ (three exponential model)

12   where f is the function that describes the change in serum concentration over time (x). A, B and D are

13   mathematical coefficients; α and δ are the rate constants for the distribution phases; β is the rate

14   constant for the terminal elimination phase.

15   The plasma curve of FB1 obtained for each animal after a single oral dose was fitted to the following

16   exponential equation:

17     $f= A*exp(-\alpha*x) + B*exp(-\beta*x) - C*exp(-K_a*x)$

18   where f is the function that describes the change in serum concentration over time (x). A, B and C are

19   mathematical coefficients; α is the rate constant for the distribution phase, β is the rate constant for the

20   terminal elimination phase and k_a is the first-order rate constant of the absorption.

21   Toxicokinetic parameters after IV (table1) and oral (table 2) dosing were determined according to the

22   following equations for each animal and expressed as mean +/- SE.

23   Area under the concentration–time curves (AUC) was calculated using the following formula:

24     $AUC=(A/\alpha)+(B/\beta)$ (IV dosing, two exponentials model)

25     $AUC=(A/\alpha)+(D/\delta)+(B/\beta)$ (IV dosing, three exponentials model)

26     $AUC=(A/\alpha)+(B/\beta)-(C/ka)$ (oral dosing)

27   Oral bioavailability (F) was determined as follows:

28     $F=AUC_{oral}/10*AUC_{IV}$ (the AUC_{IV} used was the mean obtained for three exponential model, 10 is the

29   ratio between the doses used by the oral and the iv routes)

30   Total serum clearance (Cl) was calculated as follows:

1     $Cl=dose_{(mg/kg)}/AUC$ (IV dosing)

2     $Cl=dose_{(mg/kg)}*F/AUC$ (oral dosing)

3   Mean residence time (MRT) was calculated using the following equations:

4     $MRT=(A/\alpha^2+B/\beta^2)*(1/AUC)$ (IV dosing, two exponentials model)

5     $MRT=(A/\alpha^2+D/\delta^2+B/\beta^2)*(1/AUC)$ (IV dosing, three exponentials model)

6     $AUC=(A/\alpha^2+B/\beta^2-C/ka^2)*(1/AUC)$ (oral dosing)

7   Apparent volume of distribution (Vd_{area}) was calculated as follows:

8     $Vd_{area}=Cl/\beta$ (all cases)

9   Volume of distribution in the central compartment (Vc) was calculated as follows:

10     $V_c =dose(mg/kg)/(A+B)$ (IV dosing, two exponentials model)

11     $V_c =dose(mg/kg)/(A+D+B)$ (IV dosing, three exponentials model)

12     $V_c =F*dose(mg/kg)/(A+B-C)$ (oral dosing)

13   Volume of distribution at steady state (Vd_{ss}) was determined as follows:

14     $V_{ss}=MRT*Cl$ (all cases)

15   Maximum drug serum concentration (C_{MAX}) after oral administration and the time at which C_{MAX} was

16   achieved (T_{MAX}) was determined directly from the concentration versus time curve.

17   Mean absorption time (MAT) was calculated by use of the following equation (Riegelman and Collier,

18   1980):

19     $MAT=MRT_{(oral)}-MRT_{(IV)}$ (the mean values of the respective MRT were used)

20

21   Statistical Analysis

22   Data for all response variables were reported as means +/- SE. The parameters obtained after iv

23   dosing by using the two- and the three-compartment models were subjected to 1-way ANOVA.

24   Significant differences ($P<0.05$) were reported with an asterisk.

25

26   **Results**

27   Neither mortality nor signs of pathology were observed in any of the turkeys during the toxicokinetic

28   study.

1   The elimination of FB1 from serum after intravenous (iv) dosing is presented in Figure 1. FB1

2   concentration decreased gradually from the time of administration to the end of the study. Twenty

3   hours after dosing it was below the limit of quantification (LOQ).

4   The curve obtained suggests that elimination of FB1 from the serum of turkeys can be fitted according

5   to a bi or a tri-exponential equation [Table 1, f1= A*exp(-α*x) + B*exp(-β*x), and f2= A*exp(-α*x) +

6   D*exp(-δ *x) + B*exp(-β*x)]. The functions (f) describe the change in serum concentration of FB1 at

7   any time (x); A, B and D were the intercepts at the y axis obtained from the semi-logarithmic plot of

8   serum concentration against time, and α, β and δ were the rate constants of the exponential

9   components of the curve. The very high coefficient of correlation (R^2) obtained for the two models,

10   0.9979 and 0.9992 respectively, revealed that both models are representative of the elimination of

11   FB1 from serum. Both models indicate a very rapid distribution phase (α) followed by a slower terminal

12   phase of elimination. The initial half-life of distribution (T1/2 α) was comparable in the two models

13   used: 1.7 and 3.5 min respectively. In contrast, significant differences were obtained for the half-life of

14   elimination (T1/2 β) depending on the model used. These differences were due to taking a further

15   compartment into account in the tri-exponential model. This compartment was reached by the toxin at

16   a half-life of 15.6 min (T1/2 δ). Using the two and the three compartmental models, the half-life of

17   elimination was respectively 21.3 and 85.2 min

18   The area under the curve (AUC) was close in the two and the three exponential models (respectively

19   1158 and 1390 µg/ml/min). This enabled determination of the serum clearance (Cl) of FB1, which

20   varied from 8.7 to 7.6 ml/min/kg depending on the model used, but this difference was not significant.

21   By contrast, the mean residence time (MRT) significantly differ depending on the model used (25 and

22   52 min for the 2 and the 3 compartment models respectively). The apparent volume of the central

23   compartment (V_c) did not significantly differ depending on the model used. It was low in each case,

24   about 0.1 % of the body weight (84 and 111 ml/kg for the 2 and the 3 compartment models

25   respectively). By contrast, the apparent volume of distribution (Vd_{area}) varied significantly depending on

26   the model used. It was near 0.3% of the body weight with the 2 exponential model but greater than 0.9

27   % with the 3 exponential model. No significant difference was observed for the volume of distribution

28   at the steady stage (Vd_{ss}), which varied from 0.2% to nearly 0.4% using the two and the three

29   compartments model respectively. Analysis of tissue samples collected at the end of the kinetic

1   studies was performed (table 3). Liver and kidney contained the highest levels of FB1, whereas the

2   amount of FB1 in serum and muscles was below the limit of detection.

3   The kinetic of FB1 in serum was also studied after oral dosing. As shown in Figure 2, the plot of serum

4   concentrations vs. time revealed that FB1 reached the maxima serum concentration (T_{MAX}) 3 hours

5   after oral dosing (table 2) and the toxin remained detectable 10 hours after its administration. The

6   kinetic of FB1 concentration in serum after oral dosing was fitted according to the following equation:

7    $f= A*exp(-\alpha*x) + B*exp(-\beta*x) -C*exp(-K_a*x)$. The function (f) describes the change in serum

8   concentration of FB1 at any time (x); B is the intercept at the y axis obtained from the semi logarithmic

9   plot of serum concentration against time during the terminal process, and β the rate constants of the

10   exponential that fits this process. The coefficient of correlation (R^2) obtained for this model (R^2 =

11   0.9785) suggests that it is representative of the kinetic of FB1 in serum. Determination of the area

12   under the curve (AUC) enabled estimation of the amount of FB1 that reached the serum. Mean

13   bioavailability (F) was calculated by determining the ratio between the AUC obtained with the oral

14   route and the AUC obtained after IV dosing for the 3 exponentials model. F was 3.2%, which indicates

15   that systemic absorption of FB1 is weak. The half-life of absorption (T1/2 K_a) was 44 min and the half-

16   life of elimination (T1/2 β) was 214 min. The mean absorption time (MAT) was 356 min and the MRT

17   408 min. The serum clearance (Cl) of FB1 was 7.5 ml/min/kg. The apparent volume of the central

18   compartment represented about 0.1% of body weight (111 ml/kg) and the apparent volume of

19   distribution (Vd_{area}) was nearly 2.3% of body weight. Tissue samples collected at the end of the kinetic

20   studies were analyzed (table 3). As observed after the administration of FB1 by the IV route, liver and

21   kidney contained the highest levels of FB1, the amount of FB1 in muscle being lower than the

22   concentration of the toxin in serum.

23   A complementary study was conducted on tissue contamination by FB1 in turkey poults after

24   distribution of a diet contaminated by fumonisins over a period of nine weeks. Neither mortality nor

25   signs of pathology were observed in any of the turkeys during this study, and no effect of fumonisins

26   was observed on body weight gain and the average feed intake. An interval of 8 hours was kept

27   between the last ingestion of feed and killing of the animals so as to be representative of conditions of

28   contamination that occur in the course of commercialization of turkeys. The concentrations of FB1

29   obtained in liver, kidney and muscle are listed in Table 4. As observed in the toxicokinetic studies, liver

30   and kidney contained the highest levels of FB1, whereas no FB1 was detected in muscle. The mean

1   levels of FB1 in liver were 117, 44, and 32 µg/kg in turkeys fed respectively 20, 10 and 5 mg

2   FB1+FB2/kg feed. In kidneys, traces of FB1 (22 µg/kg) were only found in turkeys fed 20 mg

3   FB1+FB2/kg feed.

4

5   **Discussion**

6   Fumonisin B1 (FB1) is a mycotoxin found all over the world in corn and corn screenings. It was

7   reported to be carcinogenic in rodents (Howard et al., 2001; Gelderblom et al., 2001), but evidence for

8   the carcinogenicity of fumonisins in humans was considered as "inadequate" (Group 2B) by the IARC

9   in 2002 (IARC, 2002). The JECFA allocated a provisional maximum tolerable daily intake (PMTDI) for

10 fumonisins (B1, B2, and B3, alone or in combination) of 2 µg/kg bw per day on the basis of short-term

11 and long-term studies of toxicity in rodents (JECFA, 2001). Because it was reported that FB1 is weakly

12 absorbed, rapidly excreted and little retained in tissues, the joint committee of the FAO and the OMS

13 concluded that "fumonisins residues in food products derived from animals are insufficient to make

14 them injurious to consumers" (EHC, 2000). On this basis, together the FDA and the EU set maximum

15 recommended levels of fumonisins in animal feed taking into account the toxicity of fumonisins in

16 these species (FDA, 2001; J.O. U.E., 2006). In this study, we demonstrated for the first time that the

17 distribution of a diet containing the maximum European tolerance for fumonisins in avian feeds (20 mg

18 FB1 +FB2/kg), does not prevent from FB1 contamination of food products derived from turkeys. This

19 experimental work strengthens results obtained during a study of food safety in France showing that

20 giblets are the most contaminated tissues by FB1 (Collectif INRA-DGAl, 2004).

21 FB1 serum kinetics after IV administration in turkeys was adequately described by a 2- or a 3-

22 compartment open model. The 2-compartment model gives a short half-life of elimination (T $_{1/2}$ β of 21

23 min). This agrees with the studies conducted in rodents and cows that reported a T $_{1/2}$ β below or

24 around 30 min (Shephard et al., 1992b,c; Prelusky et al., 1995). By contrast, the use of the 3-

25 compartment model gives a T $_{1/2}$ β of 85 min, which agrees with studies conducted in rodents and pigs

26 (Martinez-Larranaga et al., 1999; Prelusky et al., 1994). Intermediate values were reported in monkeys

27 and laying hens (Shephard et al., 1994; Vudathala et al., 1994). The clearance of FB1 in turkeys was

28 not dependant on the number of compartments used to fit the results. The value obtained (around 8

29 ml/min/kg) was relatively high in comparison with the value obtained in laying hens (1.2 ml/min/kg), but

30 very near the values observed in rat and in pig (7.2 and 9.1 ml/min/kg respectively) (Martinez-

1 Larranaga et al., 1999, Prelusky et al., 1994). The volume of the central compartment (V_c) was small,

2 in agreement with all other studies (Vudathala et al., 1994; Martinez-Larranaga et al., 1999). In

3 contrast, the apparent volume of distribution (Vd_{area}) varied from 0.3 to 1 l/kg depending on the model

4 used. The Vd_{area} reported in the literature for FB1 usually varies between 0.1 and 0.3 l/kg (Vudathala

5 et al., 1994; Martinez-Larranaga et al., 1999). For a xenobiotic, a volume of distribution of around 0.2

6 l/kg suggests that the distribution of the compound is weak, principally extracellular, with no fixation to

7 a receptor. However, studies conducted using [14C]FB1 demonstrated that FB1 is widely distributed

8 within the body, including red blood cells and the brain (Norred et al., 1993; Shephard et al., 1995).

9 Moreover, the main target of FB1 toxicity is the ceramide synthase enzyme that is localized within the

10 cell (EHC, 2000). A high Vd_{area} also agrees with studies demonstrating accumulation of FB1 in the liver

11 and kidney (Norred et al., 1993, Prelusky et al., 1996, Meyer et al., 2003; Riley and Voss, 2006; Fodor

12 et al, 2008). Finally the use of the 3-compartment model after IV dosing of FB1 in turkeys seems to be

13 more representative of FB1 persistence and toxicity than the 2-compartment model.

14 When FB1 was administrated by the oral route, maximum serum concentration was obtained 3 h after

15 dosing. This delay is longer than that obtained in other toxicokinetic studies (Vudathala et al., 1994;

16 Shephard et al., 1995; Martinez-Larranaga et al., 1999). It can be explained by the administration of

17 the toxin with feed during a meal. The amount of absorbed FB1 was low, around 3.2%, in agreement

18 with literature data (Prelusky et al., 1994 Shephard et al., 1995; Martinez-Larranaga et al., 1999;

19 Fodor et al., 2008). The mean residence time (MRT) was higher than that obtained after IV dosing and

20 the mean absorption time (MAT) was long (356 min), demonstrating that the absorption continued

21 after the maximum serum FB1-concentration was reached. These results are very close to the only

22 results published in the literature for the rat (Martinez-Larranaga et al., 1999). They agreed with the

23 presence of FB1 in liver and kidney several hours after its administration (table 3 and 4). Interestingly,

24 the clearance of FB1 obtained after its administration by the oral route is very close to that obtained

25 after IV dosing (7.5 and 7.6 ml/min/kg respectively). Because this parameter is only representative of

26 the elimination of the toxin from the body (not its absorption or distribution) these results strengthen

27 each other.

28 In conclusion, these studies revealed that FB1 was weakly absorbed in turkeys, in agreement with all

29 the data obtained in other species. It also revealed that the MRT of FB1 is relatively high after its oral

30 administration in turkeys. This result is in agreement with the demonstration of the presence of FB1 in

1   liver and kidney of turkeys eight hours after the ingestion of a diet contaminated with 20 mg

2   FB1+FB2/kg. Although the amount of FB1 in tissues is low in comparison with the high levels that can

3   be observed in maize, these results suggest that the possible ingestion by humans of fumonisins in

4   food products derived from animals should be take into account for the determination of the total daily

5   intake.

6

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1   **Legend of illustrations**

2

3   **Figure 1.** Plot of serum concentration levels vs. time in turkeys dosed intravenously with FB1 (10

4   mg/kg b.w.). Mean +/- SD, n = 6. LOQ: limit of quantification = 0.025 µg/mL

5

6   **Figure 2.** Plot of serum concentration levels vs. time in turkeys dosed orally with FB1 (100 mg/kg

7   b.w.). Values are presented as mean +/- SE, n = 6. LOQ: limit of quantification = 25 µg/L

8

9   **Table 1.** Toxicokinetic parameters of FB1 in turkey serum obtained after administration of a single

10   dose of FB1 by the intravenous route (10 mg FB1/kg bw)

11   <Table>

12   [a] $f = A \exp(-\alpha x) + B \exp(-\beta x)$

13   [b] $f = A \exp(-\alpha x) + D \exp(-\delta x) + B \exp(-\beta x)$

14   A, B, D: mathematical coefficients; α: rate constant for the quick distribution phase; β: rate constant for

15   the terminal elimination phase; δ: rate constant for a second distribution phase; $T_{1/2} \alpha$: quick

16   distribution half-life; $T_{1/2} \beta$: terminal elimination half-life; $T_{1/2} \delta$: second distribution half-life; AUC: area

17   under serum concentration-time curve from t = 0 to infinity.; MRT: mean residence time; Cl: total

18   serum clearance; Vd_{area} : volume of distribution; V_c: volume of the central compartment; Vd_{ss}: volume

19   of distribution at the steady state; Values are expressed as mean +/- SE, n = 6.

20

21   **Table 2.** Toxicokinetic parameters of FB1 in turkey serum obtained after administration of a single

22   dose of FB1 by the oral route (100 mg FB1/kg bw in feed)

23   <Table>

24   Data were modeled according to the following equation: $f = A \exp(-\alpha x) + B \exp(-\beta x) - C \exp(-K_a x)$

25   A, B, C: mathematical coefficients; α: hybrid rate constant; β: rate constant for the terminal elimination

26   phase; K_a: first order absorption rate constant; T_{MAX}: time of occurrence of maxima concentration of

27   FB1 in serum.; C_{MAX}: concentration maxima of FB1 in serum.; $T_{1/2} \alpha$: half-life at α ; $T_{1/2} \beta$: terminal

28   elimination half-life; $T_{1/2} K_a$: absorption half-life; AUC: Area under serum concentration-time curve

29   from t = 0 to infinity.; F: Extent of systemic absorption based on the determination of the ratio between

30   AUC obtained after oral administration (Table 1, 1338 µg/ml/min) and the AUC obtained following the

1 oral administration corrected by the dose used; MRT: mean residence time; MAT: mean absorption

2 time; Cl: total serum clearance; Vd $_{area}$: volume of distribution; V_c: volume of the central compartment

3 Values are expressed as mean +/- SE, n = 6.

4

5

6 **Table 3.** Concentrations of FB1 observed in the serum, liver, kidney and muscle of turkeys at the end

7 of the toxicokinetic studies

8 <Table>

9 [a] Mean +/- SE obtained 24 hours after administration of FB1 by the intravenous (IV) route (10 mg

10 FB1/kg bw).

11 [b] Mean +/- SE obtained 10 hours after administration of a single dose of FB1 by the oral route (100mg

12 FB1/kg bw in feed).

13 LD: Limit of detection: 13 µg/kg

14

15 **Table 4.** FB1 concentrations in the serum, liver, kidney and muscle of turkeys obtained 8 hours after

16 the last administration of a diet that contained different levels of fumonisins over a period of 9 weeks

17 <Table>

18 Values are expressed as mean +/- SE (n = 6)

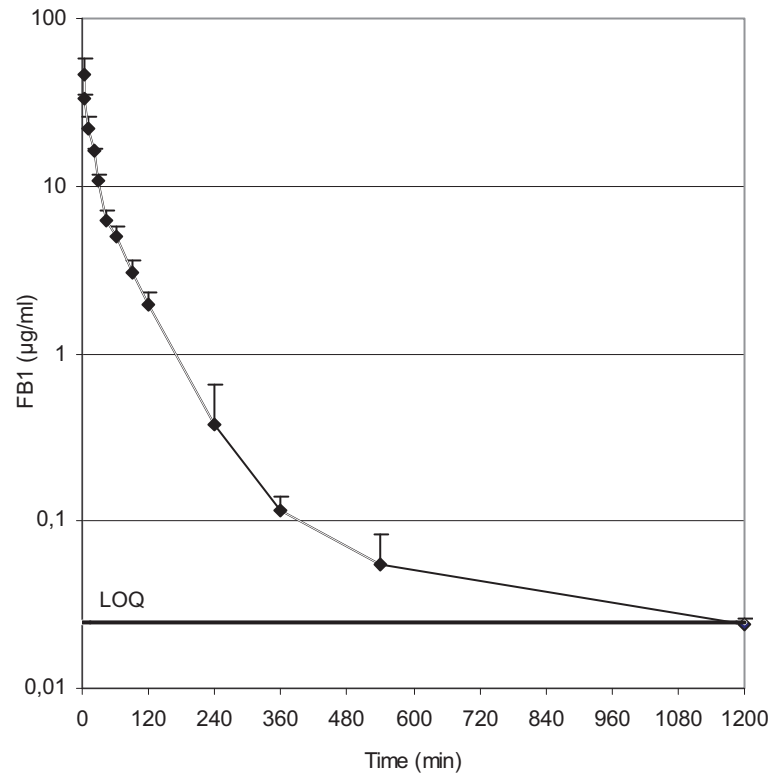19 LD: Limit of detection: 13 µg/kg

20

21

Figure 1. Plot of serum concentration levels vs. time in turkeys dosed intravenously with FB1 (10 mg/kg b.w.). Mean +/- SD, n = 6. LOQ: limit of quantification = 0.025 µg/mL
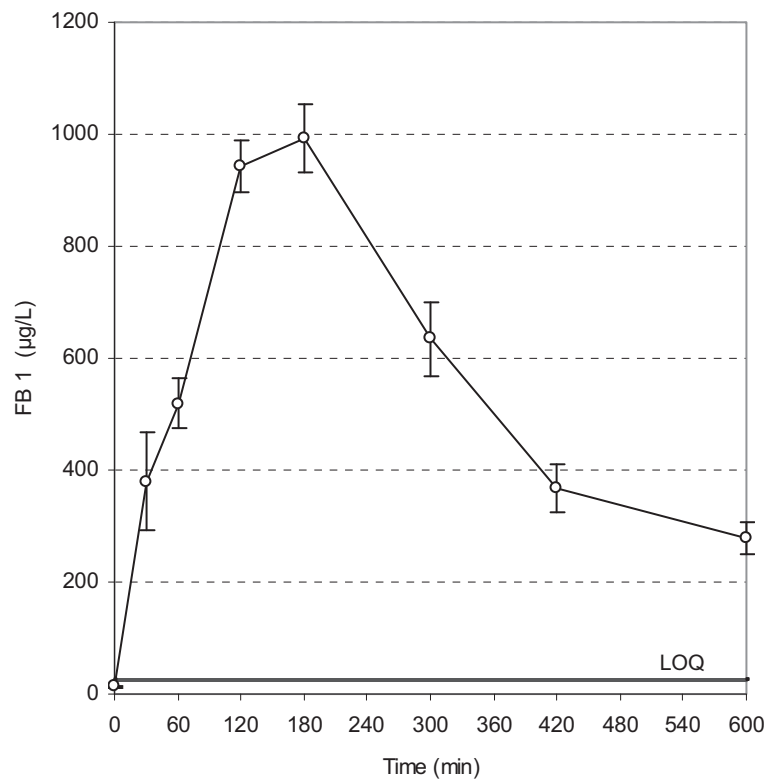
Figure 2. Plot of serum concentration levels vs. time in turkeys dosed orally with FB1 (100 mg/kg b.w.). Values are presented as mean +/- SE, n = 6. LOQ: limit of quantification = 25 µg/L

Table 1. Toxicokinetic parameters of FB1 in turkey serum obtained after administration of a single dose of FB1 by the intravenous route (10 mg FB1/kg bw)

Parameter	Value	
	2 exponentials [a]	3 exponentials [b]
A (µg/ml)	174 +/- 63	240 +/- 65
α (min^{-1})	0.6 +/- 0.2	0.6 +/- 0.1
D (µg/ml)	-	26.2 +/- 1.6
δ (min^{-1})	-	0.052 +/- 0.005
B (µg/ml)	31.9 +/- 4.1	4.3 +/- 0.5 *
β (min^{-1})	0.036 +/- 0.006	0.0084 +/- 0.0003 *
T $_{1/2}$ α (min)	1.7 +/- 0.6	3.5 +/- 0.8
T $_{1/2}$ δ (min)	-	15.6 +/- 1.9
T $_{1/2}$ β (min)	21.3 +/- 3.2	85.2 +/- 4 *
AUC (µg/ml/min)	1158 +/- 33	1390 +/- 80
MRT (min)	25 +/- 5	52 +/- 0.9 *
Cl (ml/min/kg)	8.7 +/- 0.2	7.6 +/- 0.4
Vd$_{area}$ (ml/kg)	269 +/- 47	943 +/- 81 *
V$_c$ (ml/kg)	84 +/- 32	111 +/- 22
Vd$_{ss}$ (ml/kg)	219 +/- 45	392 +/- 24

[a] $f=A*exp(-α*x)+B*exp(-β*x)$
[b] $f= A*exp(-α*x)+ D*exp(-δ*x)+B*exp(-β*x)$
* Significantly different (p<0.05, ANOVA) from the value obtained with the 2 exponentials equation
A, B, D: mathematical coefficients; α: rate constant for the quick distribution phase; β: rate constant for the terminal elimination phase; δ: rate constant for a second distribution phase; T $_{1/2}$ α: quick distribution half-life; T $_{1/2}$ β: terminal elimination half-life; T $_{1/2}$ δ: second distribution half-life; AUC: area under serum concentration-time curve from t = 0 to infinity.; MRT: mean residence time; Cl: total serum clearance; Vd$_{area}$: volume of distribution; V$_c$: volume of the central compartment; Vd$_{ss}$: volume of distribution at the steady state; Values are expressed as mean +/- SE, n = 6.

Table 2. Toxicokinetic parameters of FB1 in turkey serum obtained after administration of a single dose of FB1 by the oral route (100 mg FB1/kg bw in feed)

Parameter	Value
T_{MAX} (min)	180
C_{MAX} (µg/ml)	0.991 +/- 0.061
A (µg/ml)	10.2 +/- 1
B (µg/ml)	6.3 +/- 1
C (µg/ml)	16.5 +/- 1,9
α (min^{-1})	0.113 +/- 0.032
β (min^{-1})	0.0054 +/- 0.0004
K_a (min^{-1})	0.024 +/- 0.003
$T_{1/2}$ α (min)	29.4 +/- 3.3
$T_{1/2}$ β (min)	214 +/- 36
$T_{1/2}$ Ka (min)	44 +/- 4
AUC (µg/ml/min)	443 +/- 32
F (%)	3.2 +/- 0.2
MRT (min)	408 +/- 43
MAT (min)	356
Cl (ml/min/kg)	7.5
Vd_{area} (l/kg)	2313 +/- 388
V_c (ml/kg)	111 +/- 21

Data were modeled according to the following equation: f= A*exp(-α*x) + B*exp(-β*x) - C*exp(-Ka*x) A, B, C: mathematical coefficients; α: hybrid rate constant; β: rate constant for the terminal elimination phase; K_a: first order absorption rate constant; T_{MAX}: time of occurrence of maxima concentration of FB1 in serum.; C_{MAX}: concentration maxima of FB1 in serum.; $T_{1/2}$ α: half-life at α ; $T_{1/2}$ β: terminal elimination half-life; $T_{1/2}$ Ka: absorption half-life; AUC: Area under serum concentration-time curve from t = 0 to infinity.; F: Extent of systemic absorption based on the determination of the ratio between AUC obtained after oral administration (Table 1, 1338 µg/ml/min) and the AUC obtained following the oral administration corrected by the dose used; MRT: mean residence time; MAT: mean absorption time; Cl: total serum clearance; Vd_{area}: volume of distribution; V_c: volume of the central compartment Values are expressed as mean +/- SE, n = 6.

Table 3. Concentrations of FB1 observed in the serum, liver, kidney and muscle of turkeys at the end of the toxicokinetic studies

	IV route [a]	oral route [b]
Plasma (µg/L)	< LD	279 +/- 30
Liver (µg/kg)	46 +/- 2	5 458 +/- 509
Kidney (µg/kg)	50 +/- 1	5 785 +/- 1002
Muscle (µg/kg)	< LD	113 +/- 15

[a] Mean +/- SE obtained 24 hours after administration of FB1 by the intravenous (IV) route (10 mg FB1/kg bw).

[b] Mean +/- SE obtained 10 hours after administration of a single dose of FB1 by the oral route (100mg FB1/kg bw in feed).

LD: Limit of detection: 13 µg/kg

Table 4. FB1 concentrations in the serum, liver, kidney and muscle of turkeys obtained 8 hours after the last administration of a diet that contained different levels of fumonisins over a period of 9 weeks

	FB1+ FB2 (mg/kg of feed)			
	0	5	10	20
Plasma (µg/L)	<LD	<LD	<LD	53 +/- 13
Liver (µg/kg)	<LD	33 +/- 30	44 +/- 20	117 +/- 50
Kidney (µg/kg)	<LD	<LD	<LD	22 +/- 8
Muscle (µg/kg)	<LD	<LD	<LD	<LD

Values are expressed as mean +/- SE (n = 6)
LD: Limit of detection: 13 µg/kg