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NERVE CONDUCTION VELOCITY AND SPINAL REFLEXES MAY CHANGE IN RATS AFTER FUMONISIN B_1 EXPOSURE*

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Mycotoxin fumonisin B_1 (FB_1) a natural inhibitor of ceramide synthase contaminating mainly the corn-based food and feed may cause dysfunctions in the nervous system. In the present study peripheral neural dysfunctions were biomonitored after dietary FB_1 exposure in rats. Daily oral doses of 6.2 mg/kg body weight/day FB_1 were applied in rats for 2 weeks. Before and after FB_1 treatment nerve conduction velocities of tibial and sciatic nerves and spinal reflexes were analyzed *in vivo.* Electrophysiological recordings of biphasic plantar EMG (M and H components) and evaluation of sensory and motor nerve conduction velocities were carried out. Nerve conduction velocities revealed decreasing tendencies after FB_1 exposure. The flexor reflex and the H-components of the extensor reflex were significantly reduced. The proposed *in vivo* biomonitoring can reveal functional impairment of the peripheral nervous system caused by mycotoxin exposure. Reduction of conduction velocity and altered reflexes after FB_1 exposure are suspected to be associated with modified signal transmission due to toxic systemic effects and possible changes in sphingolipid metabolism.

Keywords: Fumonisin B_1 – nerve conduction velocity – plantar EMG – extensor reflex – flexor reflex

INTRODUCTION

Fumonisin B_1 a natural inhibitor of ceramide synthase, contaminating mainly the corn-based food and animal feed may cause dysfunctions in the nervous system. The fumonisin group of mycotoxins was first isolated and characterized in 1988 in South Africa [14] as secondary metabolites of mold *Fusarium moniliforme* growing on corn worldwide. FB_1 is the most important member of the group and, although poorly absorbed from the gastrointestinal tract, its action is at the cellular level affecting sphingolipid metabolism. Ceramides derived from sphingosine metabolism are cell regulatory factors. Because FB_1 has a close molecular resemblance to sphinganine, it interferes with ceramide biosynthesis. FB_1 plays an important role in animal mycotoxicosis and, by implication, in human disease. A more positive aspect is that fumonisins are used for elucidating the role of sphingolipids in cellular regulation [5].

* Dedicated to Professor György Ádám on the occasion of his 80th birthday.

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Fusarium moniliforme was the predominant fungus isolated from moldy corn implicated in a field outbreak of equine leukoencephalomalacia (ELEM) in South Africa in 1970 [13, 14]. Fumonisins has been shown to cause pathological changes in different organs, esophageal cancer in humans, swine pulmonary edema, liver and kidney damages in rats [8, 27], and they has become one of the five most important contaminants in terms of human exposure [1]. FB_1 does not cross the placenta and is not teratogenic *in vivo* in rats, mice or rabbits, but is embryotoxic at high, maternally toxic doses, and can damage fetuses in pregnant sows [27, 28]. Until now there are few data concerning effects of fumonisins on the nervous system *in vivo* [2, 6, 10, 14, 23]. A fumonisin B_1-induced imbalance in some brain transmitters and their metabolites were described in mice and rats [17, 25]. Fumonisins applied *in vitro* may result in neuronal malformation, disrupted axonal outgrowth, disturbances in axonal branching, visible differences in the morphology, neural tube defects or dose-dependent decrease in the survival of neurons [4, 7, 9, 21, 22]. FB_1 impairs myelin formation in aggregating brain cell culture [16]. Application of FB_1 results in a decrease of the ganglioside content and in a reduction of nerve-growth-factor induced outgrowth of neuritic processes in SH-SY5Ytrk-A human neuroblastoma cells [19]. FB_1 leads to an inhibition of neural outgrowth from embryonic chicken spinal cord explants in a tissue culture [19]. Short-term (10 days) exposure to FB_1 may modify the proliferation or differentiation of glial cells [11]. Auditory evoked potentials recorded *in vivo* on freely moving rats after feeding a corn diet containing FB_1 for 5 days revealed a highly significant 20–60% decrease in the primary and midlatency components; cortex slices *in vitro* showed a reduced excitability both in standard artificial cerebrospinal fluid solution and in a 4-aminopyridine induced epilepsy model. Spontaneous epileptic discharges after FB_1 exposure had an increased latency, decreased frequency, longer duration and modified signal forms [2].

The neurotoxic effects of natural toxins produced by molds are usually difficult to detect in humans. Therefore, model experiments on animals and biomonitoring methods are needed for the estimation of functional impairment of the nervous system. It is now evident that FB_1 causes structural and biochemical changes in neuronal membranes that have the potential for neurotoxicity.

In the present study peripheral neural dysfunctions were biomonitored after FB_1 exposure in rats. Before and after FB_1 treatment nerve conduction velocities of tibial and sciatic nerves and spinal reflexes were analyzed *in vivo.* Electrophysiological recordings of biphasic plantar EMG (M and H components) and evaluation of sensory and motor nerve conduction velocities were carried out.

MATERIALS AND METHODS

The experiments were carried out under cloralose-urethan anesthesia on 10 adult Wistar rats of both sexes (LATI, Gödöllő, Hungary) weighing between 280 and 320 g. Animals were fixed in a stereotaxic device.

Recording of the extensor reflex

To elicit the extensor reflex, the left sciatic nerve was stimulated by means of stainless steel needle electrodes inserted near to the nerve at the sciatic notch. The electrographic response (EMG) of the plantar muscle was recorded by silver electrodes with a diameter of 3 mm fixed on the plantar surface. The reflex was also induced by exciting the tibial nerve at the ankle. Randomly delivered square-wave impulses of 0.05 ms duration and of supramaximal intensity were used to stimulate the nerves. The evoked EMG responses were recorded and stored for off-line analysis by an IBM-compatible PC equipped with an A/D-card (Labmaster).

Recording of the flexor reflex

The flexor reflex of the right hind limb was induced by stimulating the plantar skin with a train of 5 impulses. The impulses had a duration of 0.5 ms, supramaximal intensity and 500 Hz frequency. The EMG activity of the tibial anterior muscle was recorded as reflex response.

Nerve conduction velocity (NCV) and intensity of spinal reflexes (RI) was determined on the basis of the parameters of averaged EMG responses.

Calculation of NCV and RI

Nerve stimulation elicited an EMG response of two components (Fig. 1). M-wave is evoked by direct excitation of motor fibers. The second, H-wave (reflex component) is caused by the stimulation of the muscular sensory afferents. The motor NCV (MNCV) and sensory NCV (SNCV) were calculated by the following equations:

$$\text{MNCV (m/s)} = \frac{\text{distance (mm) between nerve stimulation points}}{\text{difference of M-wave latencies [Fig. 1 lat. 1–lat. 3 (ms)]}}$$

$$\text{SNCV (m/s)} = \frac{\text{distance (mm) between nerve stimulation points}}{\text{difference of H-wave latencies [Fig. 1 lat. 4–lat. 2 (ms)]}}$$

To calculate RI, EMG responses were averaged by 10 and the average was rectified. The area below the curve was determined using Origin 6.0 (Microcal Co.) software (Figs 2 and 3). The values of MNCV, SNCV and RI obtained after treatment with FB_1 were compared to those calculated from the control curves by Student's *t*-test adopting a significance level of $p<0.05$.

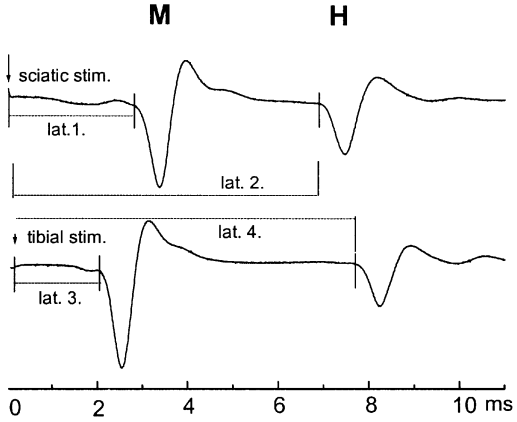

Fig. 1. Plantar EMG responses (extensor reflex) elicited by stimulation of the sciatic and tibial nerves. lat.1 and lat. 2: latency of the M and H component respectively after stimulation of the sciatic nerve. lat. 3 and lat. 4: latency of the M and H component respectively after stimulation of the tibial nerve

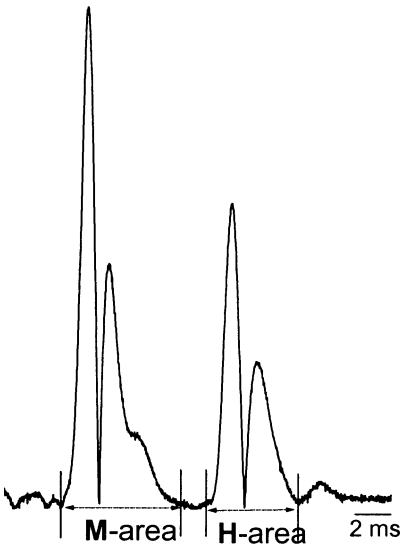

Fig. 2. Determination of the areas of M- and H-components of the extensor reflex. First, averaged EMG responses were rectified, then the area under the curves were determined

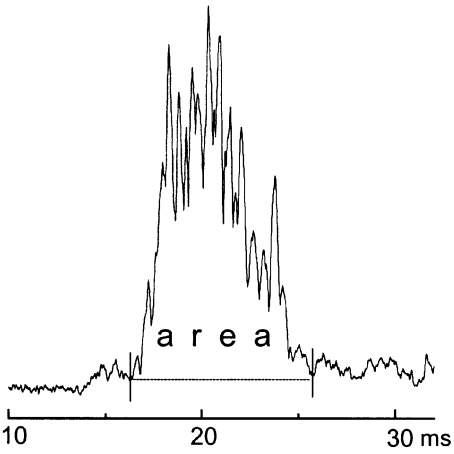

Fig. 3. Determination the area of the flexor reflex

Fumonisin B_1 exposure

In the first part of the experiments control values of the spinal reflexes were obtained from each animal. Rats were fed all the time with standard laboratory food pellets (standard laboratory chow, SLC; Bioplan CRLT/N, Hungary). They had access to food and water *ad libitum*. From our previous experiments we supposed that after a few days rats may refuse to consume the fumonisin containing feed [2]. Therefore, a soluble extract of FB_1 was prepared for FB_1 exposition. A corn material containing 4227 mg/kg FB_1 (supplied by B. Fazekas, Institute for Animal Hygiene, Debrecen, Hungary) was used to prepare the mycotoxin containing solution. Rats were gavaged daily for two weeks with the FB_1 containing saline solution to provide an exposure of 6.2 mg/kgbw/day FB_1. The necessary amount was calculated according to the measured body weight. The exact FB_1 content of saline solution was determined by HPLC (B. Fazekas, Institute for Animal Hygiene, Debrecen, Hungary). The gavaged solution with FB_1 content did not contain other mycotoxins.

FB_1 exposure was terminated after two weeks and recording of the reflexes was repeated. Control experiments on rats gavaged with the same daily amount of saline without FB_1 content were carried out in parallel experiments.

Guidelines issued by the Eötvös Loránd University's committee for the care and use of laboratory animals (Animal Experiments Protocol) was followed, in accordance to the Hungarian Animal Protection Law XXVIII/1998. The procedures used in this study complied with the humane treatment protocol.

RESULTS

Propagation of excitation induced by the stimulation was shown to spread in distal and in proximal directions as well. Thus, it was recorded as a two-component response after the stimulation of the tibial or the sciatic nerves (Fig. 1). The M-component of the evoked plantar EMG response had a shorter latency. This component was the result of direct stimulation of motor nerves, innervating plantar muscles. Thus it was caused by the excitation propagating in the distal direction. The M component was followed by the H (Hoffmann) component that had a longer latency, and was caused by monosynaptic excitation of motoneurones of the plantar muscles by afferents running into the spinal cord. Thus the H component was the result of the extensor reflex. The latency of the M component was shorter when the tibial nerve was stimulated, and longer, when the sciatic nerve was activated as seen in Fig. 1.

Rats were gavaged daily with the saline solution containing 6.2 mg/kg body weight/day FB_1. The animals were in good condition, and they showed no weight loss.

After FB_1 exposure for two weeks sensory and motor nerve conduction velocities revealed decreasing tendencies (Fig. 4). The changes were not significant at this dose of mycotoxin FB_1 application.

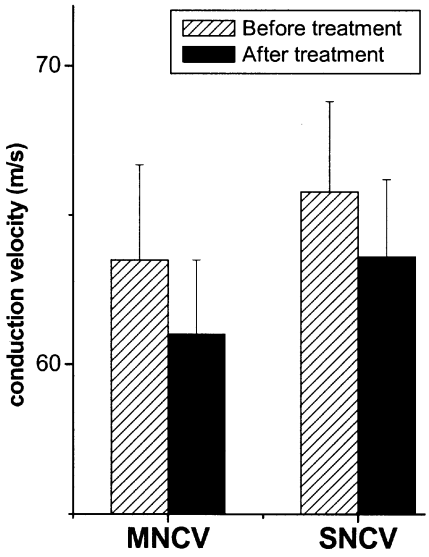

Fig. 4. Effect of fumonisin B_1 on nerve conduction velocities. MNCV: motor nerve conduction velocity. SNCV: sensory nerve conduction velocity

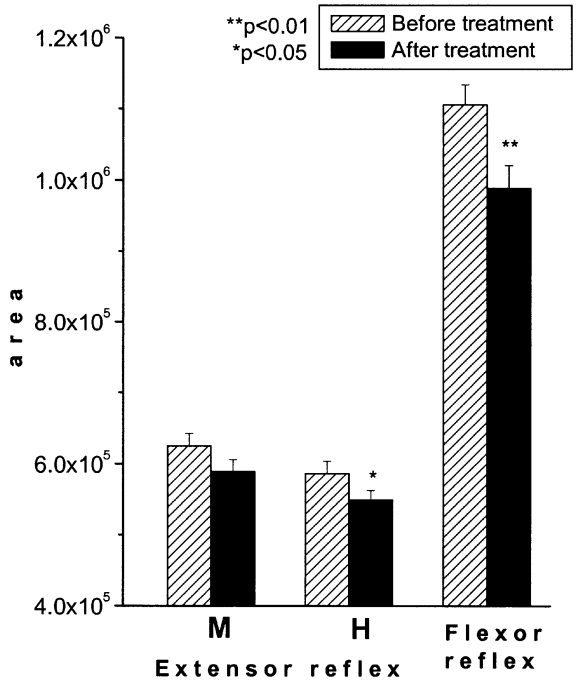

Fig. 5. Effect of fumonisin B_1 on areas of the components of the extensor and flexor reflexes

On the other hand, the intensity of the flexor reflex and the H-component of the extensor reflex were significantly reduced (Fig. 5). The H-component of the extensor reflex recorded after a two-week long FB_1 exposure revealed a significant 6.4% decrease ($p < 0.05$). The flexor reflex was reduced by 10.5% ($p < 0.01$).

DISCUSSION

FB_1, a toxin produced by *Fusarium moniliforme*, causes a variety of diseases in animals. Some of these maladies involve the nervous system, like equine leukoencephalomalacia (ELEM). ELEM was reported in equids feeding on green maize with an FB_1 content of 18.5 mg/kg (toxic level is 5 mg/kg of feed) for 2 to 3 weeks [6]. The symptoms included the disturbance of swallowing and chewing indicating the paralysis of cephalic and pharingeal muscles. In ill animals paralysis of cephalic and cervical muscles might spread to the muscles of extremities and trunk. The animals moved with difficulties, tottering and ataxia also developed. Chronic dietary exposition to the natural fungal toxin FB_1 is associated with neuronal degeneration, but identification of the cellular mechanisms underlying this neurotoxicity is difficult due to concurrent adverse systemic changes.

In our experiments no weight loss was detected in rats exposed to the mycotoxin FB_1 containing solution by oral gavage. FB_1 toxicity examined by others using gavage administration of the purified toxin to female Sprague–Dawley rats has shown similar result [3]. In this study, rats received 1, 5, 15, 35 or 75 mg/kg bw/day toxin for 11 consecutive days. Significantly depressed body weight and food consumption occurred only at the highest 35 and 75 mg/kg bw/day doses. At the same time, changes in renal morphology were observed at all doses above 5 mg/kg body wt/day. In other experiments, groups of male and female rats were gavaged daily for 14 days with doses of 0, 5, 15 or 25 mg/kg bw/day. Body weights were significantly reduced only in those male rats that received the 15 and 25 mg/kg doses [24]. Suggesting that male rats may be more sensitive to the toxin than females. These results are in agreement with our data, showing that no weight loss should be expected at the 6.2 mg/kg bw/day dose applied in our experiments. It is noteworthy, however, that in the effects detectable at higher doses of FB_1 exposure some gender differences might be expected.

At the molecular level, fumonisins inhibit ceramide synthase. Through this effect, they disrupt sphingolipid metabolism and, theoretically, sphingolipid-mediated regulatory processes that influence apoptosis and mitosis. It was shown that liver sphingolipid effects and toxicity are correlated, and ceramide synthase inhibition occurs in liver and kidney at doses below the level where any effects can be observed [27]. Treatment with FB_1 has been also shown to completely inhibit sphingosine induced neuronal differentiation [18]. Thus, available data suggest that pathological processes are mediated by altered sphingolipid biosynthesis.

FB_1 inhibits sphingolipid biosynthesis in cultured cerebellar neurons as well. It is reflected by the accumulation of free sphinganine, a reduction in the mass of total

sphingolipids and by other biochemical parameters [15]. FB_1 affects the distribution of newly synthesized ceramides among different complex sphingolipids. It has been shown that the inhibition of complex sphingolipid synthesis is reversible, and nearly normal labeling profiles can be obtained by 48 h after removing the mycotoxin. These studies establish that FB_1 inhibits *de novo* sphingolipid biosynthesis by neuronal cells, and that the limitation of ceramide synthesis differentially affects the formation of sphingomyelin. It cannot be excluded that these processes may play a role in the functional impairment of spinal reflexes.

FB_1 exposure causes marked structural and biochemical changes in the cell membranes. These altered membrane processes may be the consequences of systemic changes related to toxic effects, hepatic and renal damage [2, 3, 8, 27]. In an interesting experiment, mice received daily injections of fumonisin B_1 at doses of 0, 0.25, 0.75, 2.25 or 6.75 mg/kgbw [25]. One day after the last treatment, the brains were removed and dissected into cerebrum, cerebellum, medulla oblongata, midbrain, corpus striatum and hypothalamus. Accumulation of neurotransmitter metabolites was detected in most brain regions of the treated groups at doses comparable with ours. The revealed effects can result in an altered signal transmission in the neuronal networks.

It is reasonable to suppose that the observed changes in our experiments were due to processes induced by the FB_1 exposure. Mycotoxin FB_1 may have effect on spinal cord circuitry and may directly or indirectly influence the spinal reflexes, although the exact mechanism remains unclear. The decreasing tendencies of nerve conduction velocities may have an adverse effect on higher integrative regulatory processes of locomotion leading to pathological reactions in movement. FB_1 may affect spinal reflexes, and the deficit caused in these reflexes possibly results in locomotion problems.

In vivo biomonitoring can reveal functional damage of the nervous system potentially caused by low-dose ingestion of mycotoxins or other food-contaminants. However, *in vivo* and *in vitro* model experiments are needed in order to reveal the detailed mechanisms. FB_1, a mycotoxin produced by *Fusarium moniliforme,* causes neural dysfunctions, but little is known about the naturally occurring toxic or safe levels in foods and feeds [20]. It is impossible to keep the mycotoxins completely out of some agricultural products, but we can help to determine a tolerable level. Because of the high prevalence of FB_1 in corn in Hungary, it is necessary to extrapolate the results of animal model experiments in order to achieve a sound risk assessment of possible food-born human health hazard.

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REFERENCES

1. ApSimon, J. W., Miller, J. D. (1996) Editorial. *Nat. Toxins 4,* 1–2.
2. Banczerowski-Pelyhe, I., Világi, I., Dóczi, J., Détári, L., Kovács, F., Kukorelli, T. (2002) *In vivo* and *in vitro* electrophysiological monitoring of rat neocortical activity after dietary fumonisin exposure. *Mycopathologia 153,* 149–156.
3. Bondy, G. S., Suzuki, C. A., Mueller, R. W., Fernie, S. M., Armstrong, C. L., Hierlihy, S. L., Savard, M. E., Barker, M. G. (1998) Gavage administration of the fungal toxin fumonisin B_1 to female Sprague–Dawley rats. *J. Toxicol. Environ. Health A, 53,* 135–151.
4. de Chaves, E. I. P., Bussiere, M., Vance, D. E., Campenot, R. B., Vance, J. (1997) Elevation of ceramide within distal neurites inhibits neurite growth in cultured rat sympathetic neurons. *J. Biol. Chem*. *272,* 3028–3035.
5. Dutton, M. F. (1996) Fumonisins, mycotoxins of increasing importance: their nature and their effects. *Pharmacol. Ther*. *70,* 137–161.
6. Fazekas, B., Bajmóczy, E. (1996) Occurrence of the equine leukoencephalomalacia (ELEM) caused by fumonisin B_1 mycotoxin in Hungary. *Magyar Állatorvosok Lapja 51,* 484–487.
7. Furuya, S., Ono, K., Hirabayashi, Y. (1995) Sphingolipid biosynthesis is necessary for dendrite growth and survival of cerebellar Purkinje cells in culture. *J. Neurochem. 65,* 1551–1561.
8. Gelderblom, W. C., Abel, S., Smuts, C. M., Marnewick, J., Marasas, W. F., Lemmer, E. R., Ramljak, D. (2001) Fumonisin-induced hepatocarcinogenesis: mechanisms related to cancer initiation and promotion. *Environ. Health Perspect. 109,* Suppl. 2, 291–300.
9. Harel, R., Futerman, A. H. (1993) Inhibition of sphingolipid synthesis affects axonal outgrowth in cultured hippocampal neurons. *J. Biol. Chem*. *268,* 14476–14481.
10. Kwon, O. S., Schmued, L. C., Slikker, W. Jr. (1997) Fumonisin B_1 in developing rats alters brain sphinganine levels and myelination. *Neurotoxicology 18,* 571–579.
11. Kwon, O. S., Slikker, W. Jr, Davies, D. L. (2000) Biochemical and morphological effects of fumonisin B_1 on primary cultures of rat cerebrum. *Neurotoxicol. Teratol. 22,* 565–572.
12. Marasas, W. F. (1995) Fumonisins: their implications for human and animal health. *Nat. Toxins 3,* 193–198.
13. Marasas, W. F. (2001) Discovery and occurrence of the fumonisins: a historical perspective. *Environ. Health Perspect. 109,* Suppl. 2, 239–243.
14. Marasas, W. F., Kellerman, T. S., Gelderblom, W. C., Coetzer, J. A., Thiel, P. G., van der Lugt, J. J. (1988) Leukoencehalomalacia in a horse induced by fumonisin B_1 isolated from *Fusarium moniliforme*. *Onderstepoort J. Vet. Res*. *55,* 197–203.
15. Merrill, A. H. Jr, van Echten, G., Wang, E., Sandhoff, K. (1993) Fumonisin B_1 inhibits sphingosine (sphinganine) N-acyltransferase and *de novo* sphingolipid biosynthesis in cultured neurons *in situ. J. Biol. Chem. 268,* 27299–27306.
16. Monnet-Tschudi, F., Zurich, M. G., Sorg, O., Matthieu, J. M., Honegger, P., Schilter, B. (1999) The naturally occurring food mycotoxin fumonisin B_1 impairs myelin formation in aggregating brain cell culture. *Neurotoxicology 20,* 41–48.
17. Porter, J. K., Voss, K. A., Chamberlain, W. J., Bacon, C. W., Norred, W. P. (1993) Neurotransmitters in rats fed fumonisin B_1. *Proc. Soc. Exp. Biol. Med. 202,* 360–364.
18. Riboni, L., Prinetti, A., Bassi, R., Caminiti, A., Tettamanti, G. (1995) A mediator role of ceramide in the regulation of neuroblastoma Neuro2a cell differentiation. *J. Biol. Chem. 270,* 26868–26875.
19. Rosner, H. (1998) Significance of gangliosides in neuronal differentiation of neuroblastoma cells and neurite growth in tissue culture. *Ann. N. Y. Acad. Sci. 845,* 200–214.
20. Ross, P. F., Rice, L. G., Osweiler, G. D., Nelson, P. E., Richard, J. L., Wilson, T. M. (1992) A review and update of animal toxicoses associated with fumonisin-contaminated feeds and production of fumonisins by Fusarium isolates. *Mycopathologia 117,* 109–114.
21. Schwarz, A., Futerman, A. H. (1997) Distinct roles for ceramide and glucosylceramide at different stages of neural growth. *J. Neurosci*. *17,* 2929–2938.

22. Schwarz, A., Rapaport, E., Hirschberg, K., Futerman, A. H. (1995) A regulatory role for sphingolipids in neuronal growth. Inhibition of sphingolipid synthesis and degradation have opposite effects on axonal branching. *J. Biol. Chem*. *270,* 10990–10998.
23. Shephard, G. S., Thiel, P. G., Sydenham, E. W., Savard, M. E. (1995) Fate of a single dose of 14C-labelled fumonisin B_1 in vervet monkeys. *Nat. Toxins 3,* 145–150.
24. Tryphonas, H., Bondy, G., Miller, J. D., Lacroix, F., Hodgen, M., Mcguire, P., Fernie, S., Miller, D., Hayward, S. (1997) Effects of fumonisin B_1 on the immune system of Sprague–Dawley rats following a 14-day oral (gavage) exposure. *Fundam. Appl. Toxicol. 39,* 53–59.
25. Tsunoda, M., Dugyala, R. R., Sharma, R. P. (1998) Fumonisin B_1-induced increases in neurotransmitter metabolite levels in different brain regions of BALB/c mice. *Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol*. *120,* 457–465.
26. Visconti, A., Boenke, A., Doco, M. B., Solfrizzo, M., Pascale, M. (1995) Occurrence of fumonisins in Europe and the BRC-measurements and testing projects. *Nat. Toxins 3,* 269–274.
27. Voss, K. A., Riley, R. T., Norred, W. P., Bacon, C. W., Meredith, F. I., Howard, P. C., Plattner, R. D. (2001) An overview of rodent toxicities: liver and kidney effects of fumonisins and *Fusarium moniliforme. Environ. Health. Perspect. 109,* Suppl. 2, 259–266.
28. Zomborszky-Kovács, M., Vetési, F., Kovács, F., Bata, A., Tóth, A., Tornyos, G. (2000) Preliminary communication: examination of the harmful effect to fetuses of fumonisin B_1 in pregnant sows. *Teratog. Carcinog. Mutagen*. *20,* 293–299.