

EFFECTS OF THE TRICHOPECENES, DEOXYNIVALENOL AND T-2 TOXIN ON BRAIN
BIOGENIC MONOAMINES IN RATS AND CHICKS

by

Karen Elizabeth Boyd

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presented to the University of Manitoba
in partial fulfillment of the
requirements for the degree of
Master of Science
in
Foods and Nutrition

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MASTER OF SCIENCE

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ABSTRACT

Two experiments were conducted to determine the effects of deoxynivalenol (DON) and T-2 toxin on brain biogenic monoamines in rats and chickens. Male Sprague-Dawley rats (180 g) and 28 day-old Single Comb White Leghorn Cockerels (300 g) were orally dosed with DON and T-2 toxin at $2.5 \text{ mg kg}^{-1} \text{bw}^{-1}$. Brain biogenic monoamines were determined by high performance liquid chromatography with electrochemical detection. In the first experiment, whole brains were collected at 2, 6, 12, 24 and 48 hours post-dosing. While several interesting trends were observed, oral administration of DON and T-2 toxin did not influence whole brain concentrations of the monoamines or their metabolites in either species at any time. In the second experiment, brains were collected 24 hours post-dosing, dissected and five brain regions (pons and medulla oblongata, cerebellum, hypothalamus, hippocampus and cerebral cortex) were analyzed. DON treatment resulted in significantly ($P < 0.05$) elevated concentrations of serotonin (HT) and 5-hydroxyindole acetic acid (HIAA) in all regions of the rat. In poultry, no differences were found between the control and DON-treated animals for HT and HIAA. However, significantly lower concentrations of norepinephrine (NE) were found for the treatment group in the hypothalamus and hippocampus, and dopamine (DA) was significantly lower for the treatment group in the pons and medulla oblongata. T-2 toxin treated rats showed changes similar to those of the DON-treated rats. Values for HT and HIAA were significantly higher in the treatment group compared to the controls in all

regions. NE was also found to be significantly higher in the pons and medulla oblongata in the treatment group. In poultry, HIAA was significantly higher in the T-2 treated animals in the pons and medulla oblongata and the hypothalamus. Significantly lower concentrations of NE were found in the cerebellum and cerebral cortex in the T-2 treated animals and DA was also lower in the pons and medulla oblongata in the T-2 treated animals. These results suggest that there is an intraspecies difference in the central effects of DON and T-2 toxin.

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Chapter I

INTRODUCTION

Excessive rainfall and high humidity at harvest time in southern Manitoba in 1985 was the cause of *Fusarium* head blight on the wheat crop (Abramson et al., 1986). Similar climatic conditions in 1981 resulted in the Quebec spring wheat crop being infected with *Fusarium graminearum* (Trenholm et al., 1984). During the wheat harvest in July 1980, Ontario farmers noticed a great deal of sprouting and pink discoloration of wheat kernels (Trenholm et al., 1981). Chemical analysis of each outbreak indicated that the trichothecene deoxynivalenol was the principle mycotoxin contaminant.

T-2 toxin is endemic to Canada. Upper alimentary tract distress and necrosis of the esophagus, proventriculus, and gizzard were seen in domestic ducks, geese, horses, and swine during October 1973 in the Peace River district of northern Alberta and British Columbia (Greenway and Puls, 1976). T-2 toxin was identified as the principle contaminant in barley samples, at 25 ppm levels (Puls and Greenway, 1976). T-2 toxin is a potent mycotoxin (Ueno, 1977). A dramatic decrease in feed consumption and the loss of body weight have been observed in acutely affected animals (Weaver et al., 1978). In humans, chronic exposure to T-2 toxin causes the fatal disorder ATA, a disease that involves inflammation of the skin and mucous membranes, vomiting, leukocytopenia, and bone marrow depression (Ueno, 1977). Experimental administration of T-2

toxin results in a multisymptom syndrome, which in the acute form is characterized by hemorrhages, sepsis, and cardiopulmonary failure (Yarom et al., 1983).

In general the trichothecenes are a group of very potent toxins with DON being among the least toxic and T-2 toxin being the most toxic (Nelson, 1977). DON has been shown to cause decreased feed consumption and weight gain, diarrhea, emesis, reproductive problems, and inhibition of protein and DNA synthesis (Cote et al., 1984; McLaughlin et al., 1977). Huff et al. (1981) reported that 140 mg DON kg⁻¹bw⁻¹ caused neural disturbances similar to those seen during T-2 toxicosis. The birds began to gasp, became lethargic, and dropped their wings from the normal upright position. Subsequently, a loss of balance and righting reflex ensued. Between 3.5 and 13.5 hours death by apparent respiratory failure occurred. Autopsy revealed extensive hemorrhage throughout the carcass. It appears as though these DON induced deaths were mediated by the action of the central nervous system.

The effects of T-2 toxin have been studied most extensively in poultry. Chi et al. (1977b) reported that the clinical signs of T-2 toxicity were inactivity, diarrhea, coma, and severe oral necrosis. T-2 toxicity is characterized by the development of necrotic lesions of the mouth, crop, and gizzard; sepsis; and anorexia followed by coma and death within 48 hours (Coffin and Combs, 1981).

The literature related to the mechanism of action of T-2 toxin is limited. Wyatt et al. (1973) observed what were classified as neural aberrations, abnormal positioning of the wings, hysteroid seizures, and impaired righting reflex in young chickens fed 0 to 16 ppm T-2 toxin.

The incidence of neural disturbances depended upon the length of feeding and the concentration of T-2 toxin. Chi et al. (1981) administered 2.5 mg T-2 toxin $\text{kg}^{-1}\text{bw}^{-1}$ to chickens and assayed the whole brains for NE, DA, and HT at 4, 12, 24, and 48 hours post-dosing. Significantly higher DA concentrations were reported in the T-2-treated birds at 24 hours. NE was significantly lower at 24 hours in the treatment group. No differences were found in HT concentrations between the two groups.

Objectives:

The objectives of this study were:

1. To examine the effects of DON and T-2 toxin on the whole brain concentrations of monoamine transmitters and metabolites in rats and chicks.
2. To examine the effects of DON and T-2 toxin on the regional concentrations of brain monoamine transmitters and metabolites in rats and chicks.
3. To compare the effects of these trichothecene mycotoxins on the whole brain and regional concentrations of monoamine transmitters and metabolites in rats and chicks.

Chapter II
REVIEW OF LITERATURE

2.1 INTRODUCTION

There is a long standing history of toxicosis associated with the consumption of fungus-infected cereal grains by people and their domestic animals (Abbas et al., 1983). The contamination of crops by mold growth and the toxic metabolites that they produce continues to be an area of concern to the producers and the consumers (Abramson et al., 1983). *Fusarium* mold species are endemic to the temperate Canadian climate, and have been associated with the contamination of wheat in Quebec, Ontario, and Manitoba (Trenholm et al., 1981; Clear and Abramson, 1986).

The terms 'mycotoxin' and 'mycotoxicosis' were coined in the 1960's to categorize causative disease agents formed by toxigenic mold growth on foodstuffs and the respective illnesses attributed to animal or human consumption of such products (Wilson, 1982). Historically, the disease known as Ergotism represents the first documentation of mycotoxicosis. Ergotism is caused by the ingestion of grains infected by *Claviceps purpurea*. The fungus gains entrance into such host species as wheat, barley, oats, and, particularly, rye and triticale during flowering. Moist weather encourages spore production and ergot fungus growth, which may result in an outbreak of this disease. In man, ergotism results in the gangrenous degeneration of the lower extremities and hallucinogenic mental aberrations (Wilson, 1982).

Most recent reviews of mycotoxins begin with an account of the discovery of aflatoxin in Great Britain in 1961. Interest was aroused by the outbreak of a disease in turkeys, resulting in the loss of over 100,000 birds. At the time, its etiology was unknown so this condition was called Turkey X Disease. The cause was traced to poultry feed containing a toxic substance. This substance was found to be produced by the mold *Aspergillus flavus*, and was later named aflatoxin (Buckle, 1983).

Before 1960 and Turkey X Disease, there were sporadic reports of suspected animal poisonings by fungi in North America; however, there was no attempt to systematically study mycotoxins (Hesseltine, 1979). This has changed; in the last two decades, scores of toxic fungus metabolites have been isolated and characterized chemically and toxicologically. Many of these have been implicated in the animal disease outbreaks (Wilson, 1982).

2.2 TRICHOHECENES

2.2.1 INTRODUCTION

Fungi imperfecti of the genera *Fusarium*, *Cephalosporium*, *Myrothecium*, *Trichoderma*, and *Stachybotrys* produce a collection of toxic secondary metabolites called trichothecenes. They are characterized by a tetracyclic 12,13-epoxy-trichothec-9-ene skeleton. About 40 derivatives have been isolated, the most commonly encountered are T-2 toxin, diacetoxycirpenol (DAS), deoxynivalenol (DON), and nivalenol (Pathre and Mirocha, 1977).

Mycotoxicosis described as moldy corn toxicosis, alimentary toxic aleukia (ATA), stachybotryotoxicosis, and fescue foot disease have all been attributed to trichothecene contamination of plant products (Pathre and Mirocha, 1977). This association is based upon circumstantial evidence, that is, the clinical signs of the disease are similar to trichothecene intoxication, however, a direct link has not been demonstrated.

In addition, there has been the suggestion that trichothecenes have been used as agents in chemical warfare in Southeast Asia (Laos and Kampuchea) and Afganistan (Spyker and Spyker, 1983). *Fusarium* mycotoxins, T-2 toxin, diacetoxyscirpenol, deoxynivalenol, nivalenol, and zearalenone have been detected in samples of leaves, soil, and water, as well tissues of civilians, in the region sprayed by Soviet troops (Mirocha et al., 1983). Chemical warfare victims experienced prolonged vomiting, diarrhea, breathing difficulty, itching and skin irritation, and death. Most of the trichothecenes are potent skin irritants and inflammatory agents and could produce this toxic syndrome. The absence of any *Fusarium* mycotoxins in samples collected in areas not exposed to 'Soviet yellow rain' strongly implicates their use as warfare agents, as do the finding of T-2 toxin and HT-2 toxin in the blood, urine, and tissues of victims of these attacks (Mirocha et al., 1983).

2.2.2 OCCURRENCE

Reports of trichothecene intoxication date back to 1891. In Siberia the consumption of bread produced from molded grain resulted in a condition known as 'Taumelgetreide' or staggering grains. Clinical symptoms included vertigo, chills, nausea, vomiting, and visual disturbances in

man. When this grain was fed to livestock, feed refusal accompanied these symptoms. *Fusarium roseum*, *Gibberella saubineti*, *Helminthosporium*, and *Cladosporium herbarum* were the suspected fungi (Ueno, 1977).

In 1913, again in Siberia a serious intoxication known as 'Alimentary Toxic Aleukia' (ATA) was reported. The clinical syndrome was compatible with our knowledge of trichothecene intoxication. Outbreaks were characterized by a sudden onset of fever, hemorrhagic rash, bleeding from the nose, throat, and gums, and necrotic angina which was linked to the consumption of overwintered millet, wheat, and barley (Hesseltine, 1979). In Russia during the war years (1941-1945) ATA was a persistent problem. Conscription reduced the total manpower available to harvest crops; therefore, some grains were allowed to overwinter before harvesting. Because of general food shortages this molded grain was eaten. In 1944, over 10% of the population of Orenburg, near Siberia, died after consuming grain infected by *F. sporotrichoides* and *F. poae* (Ueno, 1977).

In 1931 and again in 1958, a type of animal intoxication known as Stachybotryotoxicosis was reported in the Ukraine and parts of central Europe. Horses, swine, cattle, and poultry were affected. The major symptoms were shock, somatitis, dermal necrosis, hemorrhage, thrombocytopenia, leukopenia, nervous disorders, and death due to respiratory failure. The disease was attributed to *Stachybotrys alterans*-molded fodder, the principle toxin of which appears to be macrocyclic dilactone derivatives of the trichothecene family (Ueno, 1977).

Intoxication due to moldy cereals was reported in European Russia during a particularly rainy summer in 1923. Weakness, vertigo, headache, and vomiting indicating an involvement of the central nervous system were caused by bread prepared from the moldy rye. Similar cases of illness associated with moldy grain were reported in the United States. Subsequent analysis isolated T-2 toxin, a trichothecene, as the causative agent (Ueno, 1977).

In Japan, Akakabi-byo (red mold disease) occurs at intervals of several years or decades, affecting large proportions of the annual national production of wheat, barley, oats, and rye. Depending upon the particular toxin involved, symptoms including vomiting and diarrhea, refusal of feed, and congestion or hemorrhage in the lung, adrenal, intestine, uterus, vagina, and brain may develop. Analysis of moldy barley has identified toxic trichothecenes such as nivalenol and fusarenon-x from *F. nivale* as the causative agent (Ueno, 1977).

The above intoxications in humans and farm animals, moldy corn toxicosis in the United States, Akakabi (red-mold) poisoning and bean-hulls poisoning in Japan, and alimentary toxic aleukia and stachybotryotoxicosis in the Soviet Union and central Europe can be attributed to mycotoxins of the 12,13-epoxytrichothecene family.

2.2.3 ENVIRONMENTAL CONDITIONS

In general, the particular fungal strains and the resulting toxic metabolites developing in moldy grain appear to be closely linked to the prevailing climate of the region. In warm climates, the development of *Aspergillus flavus* and production of aflatoxin are common. In Manitoba

and other northern temperate regions, fungi that develop at low temperatures are common. *Trichothecium roseum*, *F. roseum*, and *F. tricinctum* are commonly encountered (Hsu et al., 1972).

Studies of cultural conditions have demonstrated that each fungus can produce more than one trichothecene. The amounts of any one trichothecene produced can vary over a considerable range depending upon such factors as temperature, duration of growth, substrate, and strain of fungal species. For example, *F. tricinctum* produces primarily T-2 toxin and DAS at 8°C, but at 25°C the mold also produces HT-2 toxin, a highly potent toxin (Eppley, 1979).

Toxin contamination of foods such as peanuts and cereal grains may occur both prior to harvest and during storage as a result of improper drying or because of wetting of products not adequately protected from the weather. Fungi are particularly sensitive to weather, for mold growth to occur specific temperature and moisture conditions must be met (Tuite, 1979). Low temperatures with concomitant high moisture conditions which result in delayed harvest appear to favor mold growth (Vesonder et al., 1981). Weather can also affect the host plant making it more susceptible or less susceptible. There is an interaction between host, pathogen, and environment (Tuite, 1979).

2.2.4 STRUCTURE

There are several species of fungi known to produce trichothecenes, many of which have been implicated in episodes of toxicosis in humans and animals (Eppley, 1979). At the present time there are 43 known trichothecenes, all are produced by fungus and share the same basic

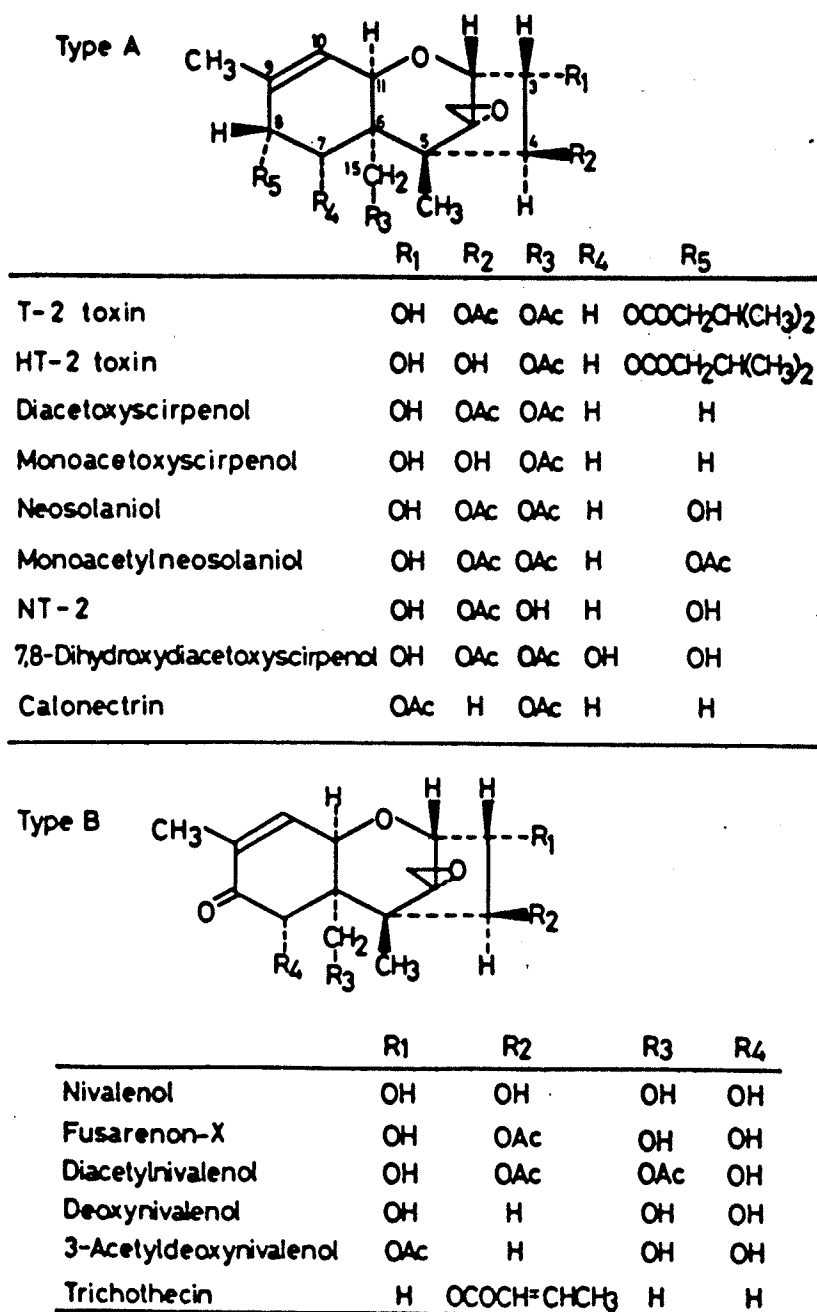


Figure 1: Chemical structures of DON and T-2 toxin and related trichothecenes (Ueno, 1977)

chemical structure. The common structure is a tetracyclic sesquiterpenoid system. The substituent (R) at positions 3, 4, 7, 8, and 15 may represent a hydrogen atom, a hydroxyl group, or an ester group (Eppley, 1979).

For convenience, the trichothecenes have been categorized according to similarity of functional groups. Ueno (1977) recognized four categories; the first is represented by T-2 toxin and diacetoxyscirpenol (DAS) (Figure 1, Type A). This is the largest category with over 20 members, 16 of which are produced by various *Fusarium* species. The second category of trichothecenes is characterized by a carbonyl function at position 8 (Figure 1, Type B). Seven of the eight members of this group are produced by various species of *Fusarium*. Deoxynivalenol is a member of this group. The third category includes verrucarol among its 12 members of the macrocyclic dilactone derivatives. These metabolites have been isolated from *Myrothecium*, *Stachybotrys*, *Verticimonosporium*, and *Cylindrocarpum*. The fourth category has only one member; crotocin. It has a second epoxide function at the 7-8 position.

2.3 DEOXYNIVALENOL

2.3.1 INTRODUCTION

The mycotoxin (3α , 7α , 15-trihydroxy-12,13-epoxy-trichothec-9-ene-8-one) was first identified in Japanese barley and given the name Rd toxin (Yoshizawa and Morooka, 1973; Vesonder et al., 1973). Two independent investigators determined the structural formula of Rd toxin and assigned the trivial names deoxynivalenol (DON) and vomitoxin. DON, along with T-2 tetraol, scirpentriol, fusarenone-x and nivalenol, belongs to the second category of trichothecenes, this group is characterized by a carbonyl function at position 8 (Eppley, 1979).

Fusarium mold species are endemic to the temperate Canadian climate, and have been associated with the DON contamination of wheat in Ontario and Quebec (Trenholm et al., 1983). In 1984 and again in 1985, analysis of Manitoba wheat that was visibly damaged by mold growth identified DON as the principle trichothecene contaminant (Clear and Abramson, 1986). Low temperatures with concomitant high moisture conditions promote the infestation of grain by *F. graminearum*. Infestation usually occurs as a result of field infection rather than as a result of post harvest development. Mold growth begins at the ear tip and is referred to as pink ear rot in corn and head blight in grain. The affected kernels are lighter in color and lower in density than normal kernels. Severely infected kernels become shriveled and pink or red colored (Pollmann et al., 1985).

Once produced, DON persists in the molded crop and treatments with various agents, bleaches, and oxidizing compounds have not been shown to reduce its level. Feed additives have not been demonstrated to successfully mask its physiological effect. While the extent of DON contamination correlates with above average rainfall at the time of flowering, disease intensity does not appear to relate to toxin concentration in the edible plant material (Miller et al., 1983). The presence of disease can lower grain quality to an extent that it should be down graded and removed from the human diet (Pollmann et al., 1985).

2.3.2 TOXICITY

Vesonder et al. (1973) isolated and characterized DON and regarded it to be a major emetic and feed refusal agent in molded grain for swine. An early study by Forsyth (1974) showed corn infected with *F. graminearum* caused feed refusal in swine and rats, and vomiting in swine.

There is some evidence suggesting that swine are particularly sensitive to DON; poultry and cattle are more resistant (Trenholm et al., 1981).

The literature regarding the physiological and pathological effects of DON is often conflicting. This is seen particularly with respect to DON's reputed emetic principle. Vomiting by swine fed moldy corn infected by *Fusarium* species was first reported in 1928. Since then, episodes of emesis resulting from consumption of other moldy cereal grains both in humans and animals have been reported periodically; however, this physiological effect is not repeatable (Vesonder et al., 1973).

Cote et al. (1984) screened 342 feed samples for mycotoxins, including DON, T-2 toxin, diacetoxyscirpenol, zearalenone, and aflatoxins. This was not a random sample; only feeds suspected of causing or contributing to animal health problems were submitted for analyses. Eighty percent of the samples were found to contain DON. Corn and corn-based mixed feed were the most common DON contaminated samples with the concentration of DON ranging from 0.1 to 41.6 ppm. The clinical signs and lesions included reduced feed consumption, chronic gastrointestinal problems, and hepatic necrosis. While the survey represents the results from analyses of suspected contaminated samples and not from samples collected at random, it appears that animal health and productivity problems are associated with DON-contaminated feeds.

These findings must be interpreted with caution since, for swine the clinical symptoms associated with DON-contaminated feeds are not unlike those associated with other diseases. Feeding trials with purified DON in levels that have been found in nature have not confirmed field observations (Vesonder and Hesseltine, 1981; Cote et al., 1984). The ques-

tion still remains whether the problems that occur in swine consuming DON-contaminated feeds are caused by: 1) DON, 2) other mycotoxins, 3) an interaction between DON and other mycotoxins, or 4) by unidentified factors.

2.3.2.1 STUDIES IN SWINE

Forsyth et al. (1977) reported almost total refusal of feed when pigs were fed a diet naturally contaminated with *G. zeae* (Table 1). DON is often accompanied by low levels of zearalenone and it is thought to have a synergistic effect. Zearalenone assay detected about 1.0 ppm in the *G. zeae* contaminated sample. Pigs fed a diet supplemented with 7.2 ppm of pure DON exhibited only a 40% reduction in feed consumption compared with almost total refusal with *G. zeae* contaminated feed.

Vesonder and Hesseltine (1981) in a retrospective study reported that in 1965 hog producers noticed that *G. zeae* infected corn was refused by swine and caused a few pigs to vomit. In feeding trials, swine rejected the naturally infected corn and vomited when administered a water extract obtained from the moldy grain via gastric intubation, or intravenous or i.p. injection. In contrast to Forsyth et al. (1977), Vesonder and Hesseltine (1981) found complete feed refusal, vomiting, and death at low DON concentrations (e.g. less than 1.0 ppm).

The results of a feeding trial by Young et al. (1983) indicated that young pigs may be more susceptible to the toxicological effects of DON than mature animals. Levels greater than 2.5 ppm resulted in a 50% reduction in growth rate and serious weight loss occurred at levels greater than 11.0 ppm. Vomiting was observed only in those pigs fed diets containing 19.7 ppm DON.

TABLE 1
STUDIES OF DON IN SWINE

Reference	Species	Route	DON (ppm)	Symptoms
Trenholm et al. (1984)	pregnant gilts	oral	3.5	feed refusal
Forsyth et al. (1977)	mature pigs	i.p.	.05 mg/kg bw	emesis
	mature pigs	oral	0.1 mg/kg bw	emesis
	mature pigs	oral	3.6	feed refusal reduced growth rate
Young et al. (1983)	piglets	oral	19.7	emesis feed refusal
	piglets	oral	1.3	feed refusal
Pollmann et al. (1985)	piglets	oral	1.0	feed refusal
	mature pigs	oral	2.8	reduced growth rate

Results of the studies by Pollmann et al. (1985) and Young et al. (1983) agree: feeding diets of less than 1.0 ppm DON did not appear to affect feed consumption, feed efficiency, body weight, Feed containing 1.3 ppm DON produced a marked depression in the performance of piglets or certain blood constituents. A diet contaminated with 1.0 ppm DON caused decreased feed consumption in the weanlings. Feed efficiency appeared to be somewhat poorer for pigs that had been on diets containing DON. Traces of DON were found in the kidney, liver, spleen, and heart tissues. No differences were observed in histopathological examination. A level of 2.8 ppm DON in the feed was reported to be the lowest concentration of DON causing significantly reduced feed intake and growth rate in mature pigs. These pigs stopped eating in the first week of the study and performed so poorly after two weeks that they were withdrawn from the experiment. The pigs consuming 2.2 ppm showed no evidence that ingestion of DON-contaminated feed had any effect on organ size or tissue structure.

Trenholm et al. (1984) investigated the nutritional and toxicologic effects of dietary DON under laboratory conditions. They reported an initial reduction in dietary intake in direct proportion to the DON content of the diet. After a few days' exposure to the contaminated feed, daily feed consumption increased. There was no indication of vomiting or serious illness in any of the pigs. Results of these trials were similar to those of Young et al. (1983) and showed that feeding pigs diets with less than 1.0 ppm DON did not affect feed consumption, feed conversion, and certain blood constituents. Chavez (1984) found that inclusion of DON-contaminated wheat in the diet of weanling pigs up to a level of 1.73 ppm did not affect piglet performance. Piglets fed a

diet containing 2.5 ppm showed a significant decrease in body weight gain. This reduction in body weight gain was the reflection of a significant reduction of feed consumption over the entire experiment. No effect on feed efficiency was reported, indicating the main effect of DON was to reduce feed consumption.

2.3.2.2 STUDIES IN POULTRY

Until recently there were no reports on the effects of DON on mortality and performance of broiler chicks (Table 2). Hulan and Proudfoot conducted their 1982 study to investigate such factors. Feeding of up to 1.87 ppm DON to the chickens had no effect on body weight gain, feed consumption, mortality, or feed efficiency.

In a more intensive study, Hamilton et al. (1985) examined the effects of feeding diets that contained wheat naturally contaminated with DON to White Leghorns, broiler chicks, and turkey poults. In general, the Leghorn chicks fed diets containing DON levels up to 4.6 ppm showed increased feed intake and daily body weight gains. Feed intake, body weight gain, feed efficiency, and mortality results for broiler chicks agreed with those of Hulan and Proudfoot (1982). The low mortality incidence of this study indicates that DON was not toxic at these dietary levels. Examination at necropsy of randomly selected chicks and poults provided no apparent evidence of lesions in the oral cavity, esophagus, proventriculus, and gizzard; of abnormalities in the spleen, heart, liver, and kidneys; or of hemorrhaging in the viscera or muscles of the birds.

TABLE 2
STUDIES OF DON IN POULTRY

Reference	Species	DON (ppm)	Symptoms
Hulan and Proudfoot (1982)	broiler chicks	1.87	no significant effects
Hamilton et al (1985)	white leghorn hens	4.6	DON fed chicks gained faster than controls
Moran et al (1982)	broiler cockerels	181	feed refusal oral and gastric lesions
Huff et al (1981)	broiler chicks	140 mg/kg bw	hemorrhage, necrosis, nervous disorders
Farnworth et al (1983)	white leghorn hens	0.35	elevated liver lipids and triglycerides
Trenholm et al (1984)	white leghorn hens broiler chicks turkey poults	5.0	no significant effects

The preceding studies suggest that chickens may be relatively tolerant to the effects of DON. Farnworth et al. (1983) conducted feeding trials on chicks giving particular attention to the liver because of the hepatotoxic effects documented for other mycotoxins. Lipid analysis revealed that the percent total lipid and triglyceride content of livers from chicks receiving 0.35 ppm of DON were significantly higher than for birds receiving the control diet. At the same time, the level of DON had no effect on growth, feed consumption, or feed efficiency. The findings of significantly increased liver lipids and triglyceride in chickens fed DON-contaminated diets indicate that DON may affect lipid metabolism. The changes in liver lipid levels were detected even though the toxin levels ingested were not high enough to affect the various growth parameters.

Moran et al. (1982), who fed broiler chicks diets that contained DON, found that growth and feed intake were not decreased until the DON content of the diet exceeded 116 ppm. Pathologic lesions observed upon necropsy were confined to birds that had received the contaminated corn. Severity and incidence increased in proportion to the dietary level of contamination. Only oral and gastric areas were involved. Lesions in the gastric area did not appear until DON contamination exceeded 116 ppm.

Huff et al. (1981) administered acute dosages of DON to broiler chickens. A single dose of 140 ppm induced extensive ecchymotic hemorrhaging throughout the carcass, widespread deposits of urates, nervous disorders, and swallowing motions indicative of upper gastrointestinal irritation. These symptoms are consistent with descriptions of the hemorrhagic anemia syndrome of chickens caused by moldy feed.

2.3.2.3 STUDIES IN RATS

In young pigs, a dietary concentration of 19.7 ppm DON caused vomiting (Young et al., 1983), 12 ppm induced almost total feed refusal (Forsyth et al., 1977), and 1.3 ppm DON produced marked depression in growth performance. Although swine are generally most sensitive to DON contaminated feeds, Vesonder et al. (1979) report that rats are responsive to DON at levels similar to those that affect swine.

Morrissey and Vesonder (1985) reported an initial reduction in feed consumption in rats fed 20 ppm DON similar to that of pigs. However, after a few days, intake and weight gains increased to control levels. The authors accredited the differences between their results and those of others (Trenholm et al., 1983) to the fact that while similar concentrations were being studied, their diet contained purified DON added to clean feed rather than the naturally contaminated grains. They suggested an involvement of other toxins such as zearalenone.

A decrease in fertility of Sprague-Dawley rats consuming 20 ppm of DON was reported compared to a control group. This effect appears to be a result of feeding male rats 20 ppm of DON for 60 days prior to breeding. Khera et al. (1984), in a similar experiment, reported no significant differences between control and DON treated groups in the number of males that induced pregnancy and females that became pregnant after mating trials. Male rats were fed the toxin for 42 days only prior to mating. One possible explanation for the difference in findings between the two studies is that a 60- to 70-day minimum exposure of male rats to the toxin is required to allow the toxin to affect all stages of spermatogenesis. Neither study reported any gross or histologic changes in testes or ovary structure.

2.4 T-2 TOXIN

2.4.1 INTRODUCTION

T-2 toxin (3 α - hydroxy- 4 β , 15-diacetoxy- 8 α - (3-methoxybutyryloxy)-12,13-epoxy-trichothec-9-ene) is a toxic metabolite produced by *Fusarium tricinatum* as well as other species of *Fusarium* (Yarom et al., 1984). *Fusarium tricinatum* is widely distributed in nature, endemic to Canada. It is reported to be most prominent on moldy corn in low temperature storage (Chi et al., 1977a). T-2 toxin is one of the most potent trichothecenes; it has been implicated in episodes of human and animal toxicosis, the most significant human outbreak being ATA in the Soviet Union (Forsell et al., 1985).

A variety of clinical, pathological, and biochemical changes have been identified in animals exposed to the mycotoxins under field conditions and in experimental studies with pure T-2 toxin. The harmful consequences of ingestion of T-2 toxin have been examined in rats, mice, poultry, and swine. T-2 toxin has been reported to cause reduced feed consumption, reduced growth rates, and usually inflammatory lesions around the mouth and in the upper alimentary tract (Hayes et al., 1980).

2.4.2 TOXICITY

One noted outbreak of T-2 toxin in Canada was reported in the Peace River District of northern Alberta and British Columbia (Greenway and Puls, 1976; Puls and Greenway, 1976). Clinical symptoms were observed in domestic ducks, geese, horses, and swine during the fall of 1973. All species showed upper alimentary tract distress with mortalities occurring in geese. Severe necrosis was reported on the membranes of

the esophagus, proventriculus, and gizzards of the geese. The only common ingredient was barley, which originated from the Peace River District. T-2 toxin was identified in the barley at a level of approximately 25 ppm.

2.4.2.1 STUDIES IN POULTRY

Wyatt et al. (1972) reported a linear increase in lesion size with increasing toxin concentration when diets of graded doses of T-2 toxin were administered to chickens (Table 3). The lesions became so severe that the birds could eat only with difficulty. This condition impaired the growth rate without affecting feed-conversion efficiencies.

Chi et al. (1977a) reported no signs of digestive disorders, hemorrhages, or pathologic changes in chicks fed 0 to 4.0 ppm purified T-2 toxin. Histopathologic examination of various organs failed to find marked pathologic changes except for necrotic lesions in the crop and gizzard of chicks fed high concentrations of T-2 toxin. Like Trenholm et al. (1984) who conducted feeding trials with purified DON, they failed to induce the serious disorders described in field reports of naturally contaminated grain. Feed consumption and weight gain of chicks fed a diet containing 4.0 ppm T-2 toxin decreased from the first week. Oral lesions were observed from the second week suggesting that painful mouth parts may cause the decrease in feed consumption and resulting decrease in weight gain in T-2 treated birds.

In another study, Chi et al. (1977b) found that T-2 toxin had detrimental effects on egg production, shell quality, and hatchability at the level of 8 ppm. Oral lesions were similar to those observed in broiler

chicks fed T-2 toxin (Wyatt et al., 1972; Chi et al., 1977a). They appeared in the second week in hens fed 4.0 and 8.0 ppm T-2 toxin. By the third week, even hens fed 0.5 ppm had small oral lesions. The severity and incidence of lesions were proportional to the amount of the toxin in the diet. Other than these observations, no pathological or clinical signs were noted in necropsy examinations.

The acute toxic effects of several 12,13-epoxytrichothecenes were investigated in one-day-old broiler chicks by single oral doses by Chi et al. (1978). The descending order of acute toxicity of the tested trichothecenes by LD₅₀ was 8-acetylneosolaniol, diacetoxyscirpenol, T-2 toxin, HT-2 toxin, neosolaniol, deacetyl-HT-2 toxin, and T-2 tetraol. The toxic potency appeared to depend upon the side chains. The results indicated that T-2 toxin was metabolized into less toxic compounds when consumed by chickens. The clinical signs of the toxin-treated birds were similar to those of earlier studies (Wyatt et al., 1972; Chi et al., 1977a, 1977b). The weight gain and feed consumption of the surviving birds treated with each toxin decreased proportionally with the amount of toxin administered.

Speers et al. (1977) found no significant effects of feeding rations containing 4.0 and 8.0 ppm purified T-2 toxin to laying hens. Only at a concentration of 16.0 ppm T-2 toxin were feed consumption, body weight gain, and egg production reduced. At 16.0 ppm T-2 toxin produced no changes in any blood parameters examined. Lesions were noted on the mandible and tongue of birds fed 8.0 and 16.0 ppm T-2 toxin. Diet containing 2.5 and 5.0% corn invaded with *F. tricinctum* was fed to hens. The diet containing 5.0% corn invaded with *F. tricinctum* drastically reduced feed consumption, body weight gain, and egg production. Lesions

TABLE 3
STUDIES OF T-2 TOXIN IN POULTRY AND SWINE

Reference	Species	T-2 toxin (ppm)	Symptoms
Wyatt et al. (1972)	male broiler chicks	16.0	oral lesions impaired growth rate
Chi et al. (1977a)	broiler chicks	4.0	no digestive disorders, hemorrhages or pathologic changes
Chi et al. (1977b)	SCWL hens	8.0	oral lesions and detrimental effects on egg production, shell quality and hatchability
Speers et al. (1977)	laying hens	8.0	no significant effects
Weaver et al. (1978)	swine	3.2 mg/kg bw	lesions

developed on the tongue and skin at the corners of the mouth and became progressively worse as the experiment continued. Analyses of feed revealed 8.0 ppm and 16.0 ppm T-2 toxin in the 2.5 and 5.0% *F. tricinctum* contaminated feeds. There were no significant changes in blood characteristics at either concentration of T-2 toxin. Thus, body weight and egg production changes appear to be the result of reduced feed intake. They reported no evidence to suggest some metabolic disturbance or inhibition may be involved.

2.4.2.2 STUDIES IN SWINE

Weaver et al. (1978) conducted acute and chronic T-2 toxicity studies on swine, from which some interesting points may be noted. Since it was found that most of the T-2 toxin associated symptoms reported previously were not produced by purified T-2 toxin in swine in a single LD₅₀ dose or when consumed orally at concentrations between 1.0 and 8.0 ppm for an eight week period, it seems probable that T-2 toxin is not the major cause of moldy corn toxicosis. In fact, T-2 toxin has not been found in nature in concentrations sufficient to produce the reported conditions; and in these cases where T-2 toxin-induced lesions in animals, most of the workers were dealing with fungus cultures rather than pure T-2 toxin.

In the acute study T-2 toxin-induced lesions were reported in only those swine that died; those swine that survived the LD₅₀ injection and those used in the chronic study were free of lesions.

2.4.2.3 STUDIES IN RATS

T-2 toxin and its related compounds have strikingly cytotoxic action in target organs such as liver, intestine, thymus, spleen, and bone marrow. Signs of acute toxicosis included loss in body weight, emesis, and inflammation (Tsuchida et al., 1984) as well as hematological changes (Chan and Gentry, 1984), destruction of bone marrow, inhibition of protein synthesis (Suneja et al., 1983), and fatty infiltration of the liver (Suneja et al., 1984). Up to now, few papers have reported the in vivo metabolism of T-2 toxin in rats. Yoshizawa et al. (1980) found orally administered T-2 toxin was eliminated in the excreta of rats as HT-2 toxin, neosolaniol, and several unknown metabolites.

T-2 toxin and related trichothecenes are well known skin irritants. This property has been applied for biological screening of fusarium spp. and their toxins (Ueno, 1984). Hayes and Schiefer (1979) reported an acute dermal inflammation reaction, characterized by hyperemia, edema, and variable degrees of necrosis of the epidermis when low doses of T-2 toxin (15 ug ml^{-1}) were applied topically to the shaved skin of rats. The reaction increased in intensity until 48 hours, after which the inflammatory response rapidly diminished.

Chan and Gentry (1984) reported an LD_{50} value of $0.85 \text{ mg kg}^{-1} \text{ bw}^{-1}$ for rats given a single intramuscular injection. The toxicosis caused significant increases in bromosulfalein retention and alanine aminotransferase activity indicating that the hepatobiliary system is a major target organ.

Suneja et al. (1984) reported on the effects of dietary T-2 toxin on lipid metabolism in rats. A significant increase in total liver lipids,

triglycerides, and free cholesterol was found in rats that consumed $1.5 \text{ mg kg}^{-1} \text{ bw}^{-1}$ of T-2 toxin for 4 consecutive days. Enlargement of the liver, which could be the result of edema, a general increase of protoplasm, or an increase of specific constituents such as lipids or glycogen, was also noted in the toxin-treated animals.

2.5 MYCOTOXINS AND THE CENTRAL NERVOUS SYSTEM

A consequence of T-2 intoxication which has received less attention is its influence on the nervous system. T-2 toxin has been implicated in 'bean-hulls poisoning' of horses (Wyatt et al., 1973). Horses fed dried bean hulls developed symptoms such as convulsions, respiratory problems, and disturbances of the nervous system (Ueno, 1977). ATA, which is generally thought to be trichothecene intoxication, has several features indicating a serious involvement of the central nervous system. These features may include impaired reflexes, meningism, various neuropsychiatric manifestations, and encephalitic symptoms (Wyatt et al., 1973).

2.5.1 STUDIES IN POULTRY

Wyatt et al. (1973) investigated some of the effects of T-2 toxin on the nervous system of chickens in an attempt to gain further information about T-2 toxin and its possible role in moldy corn toxicosis and ATA. Young chickens fed dietary T-2 toxin showed an abnormal positioning of wings, hysteroid seizures, and impaired righting reflex. The incidence of neural disturbances was dependent upon the age of the bird, as well as the dose of dietary T-2 toxin. 4.0 ppm T-2 toxin was the minimum level reported to induce abnormalities.

Huff et al. (1981) reported similar neural disturbances when intubating DON to chicks. Immediately after administration of DON, the birds began to gasp, became lethargic, and dropped their wings and head from the upright position. A significant number of hemorrhagic lesions throughout the intestinal tract, liver, and musculature were reported upon necropsy at levels of 140 to 1120 mg kg⁻¹ bw⁻¹.

In a subsequent study to gain information about the effects of T-2 toxin on the neural system of chickens Chi et al. (1981) observed the concentrations of brain catecholamines in T-2 treated chickens. After only 12 hours chickens treated with T-2 toxin showed significantly greater dopamine concentrations. The dopamine concentrations were further increased after 24 hours, and then decreased to the control level after 48 hours. Brain norepinephrine concentration was found to be significantly decreased 24 hours after administration of treatment, and remained at that level until 48 hours after dosing. There was no significant difference found in the brain serotonin between control and T-2 treated chickens.

The authors theorize that these findings may be a result of a chemical stress or of inhibition of dopamine- β -hydroxylase. The chemical stress placed on the birds by the administration of the toxin could cause a rise in temperature which in turn could cause an increase in the turnover rate of norepinephrine. There is also the possibility that T-2 toxin may inhibit dopamine- β -hydroxylase, the enzyme required for the conversion of dopamine to norepinephrine. This possibility was substantiated by the findings that the increase in dopamine concentration is in association with the decrease in brain norepinephrine concentration. A similar pattern is observed when dopamine- β -hydroxylase inhibitors are

used. The changes in catecholamine metabolism have been found with motor activity, behavior, and a variety of other conditions. However, the relationship of the changes in brain catecholamines observed in this study and neurosymptoms of DON and T-2 toxicosis observed in other studies is less clear because of the complexity of the variables in brain amine metabolism.

2.5.2 CENTRAL NERVOUS SYSTEM

The brain uses stereotyped electrical signals to process all the information it receives and analyzes. The signals are virtually identical in all nerve cells. The origins of the nerve fibers and their destinations within the brain determine the content of the information they transmit. They secrete various chemical substances called neurotransmitters, used for conveying signals from one cell to the next (Kuffler et al., 1984).

The composition and function of the brain can be altered by alterations of brain neurotransmitter systems, since neurotransmitters convey the nerve impulse across the synapse to either another neuron, a muscle cell, or a secretory cell (Wurtman, 1982). The principle neurotransmitters (norepinephrine, dopamine and serotonin) are formed by hydroxylation and decarboxylation of the amino acids phenylalanine, tyrosine, and tryptophan (Ganong, 1983).

2.5.3 EMESIS MEDIATED BY CENTRAL SYSTEMS

The nausea and vomiting reported by Forsyth (1974) and Vesonder et al. (1973) in swine consuming DON appears to have been precipitated, in this case, by a chemical stimulus. Borison et al. (1981) reported that

chemical stimuli, including various drugs, in the blood and cerebral spinal fluid activate neurons in the area postrema, the so-called chemoreceptor trigger zone, which then stimulate the lateral reticular formation at the site of the vomiting center. Similarly, visceral afferents, primarily of vagal origin, act via the nucleus tractus solitarius to stimulate the vomiting center.

The effect of a chemical stimulus is produced through one of two pathways: either through attachment of the stimulus to a receptor of a neurotransmitter or by alteration of the availability of a neurotransmitter to its receptor. Unfortunately, substances being discovered as effective neurotransmitters in the nucleus tractus solitarius are extensive, including the catecholamines, norepinephrine and dopamine, as well as the indoleamine serotonin (Borison et al., 1981).

2.5.4 THE NOREPINEPHRINE SYSTEM

Norepinephrine (NE) is converted from tyrosine in the brain and adrenal gland. This metabolism occurs in the nerve endings of the NE containing neurons of the central nervous system, in the peripheral neurons, and in the adrenal medulla (Guroff, 1980). The NE-containing cell bodies are found in ten or more clusters in the medulla oblongata, the pons and the midbrain, with the most densely packed region being in the locus coeruleus (Guroff, 1980). These cell bodies send fibers upward through the reticular activating system to essentially all parts of the diencephalon and cerebrum (Ganong, 1983). The ventromedial nuclei are areas of the hypothalamus capable of blocking such drives as eating, drinking, and sex because of its NE activation. Thus, at the same time that the NE system activates the cerebral cortex, it also

seems to block other drives that might compete with the higher levels of celebration (Guyton, 1981).

Most of the current information about the action of NE has come from pharmacological studies in which the concentration of the amine has been altered by interfering with one of the steps in its biosynthesis, its storage, its reuptake, or its degradation (Guroff, 1980). These studies indicate that mood is related to the amount of free NE available at synapses in the brain (Ganong, 1983). It is believed that overstimulation of the NE system is the cause of the manic phase of the manic-depressive psychosis. Drugs that decrease NE removal from the synapses are frequently very effective both in increasing the activity of the NE system and also in the treatment of the depressive psychoses (Guyton, 1981).

The NE containing neurons in the hypothalamus are involved in regulation of the secretion of anterior pituitary hormones, and they appear to inhibit the secretion of vasopressin and oxytocin. There is considerable evidence that NE is involved in the control of food intake and along with serotonin, it appears to be involved in the regulation of body temperature (Ganong, 1983).

2.5.5 THE DOPAMINE SYSTEM

Dopamine (DA) was originally thought to function merely as an intermediate in the biosynthesis of NE. It is now known that DA is present and has independent functions in certain distinct portions of the brain. The dopaminergic tracts originate in the nigrostriatal bundle and in the diencephalon and the telencephalon. The neurons are located in the

substantia nigra, and the axons terminate in the caudate nucleus and the putamen of the corpus striatum (Guroff, 1980).

These dopaminergic fibers also project to other regions of the brain especially into two areas of the hypothalamus (Guyton, 1981). Stimulation of this system increases the activity of the lateral hypothalamus, producing enhanced drives for eating and other types of activities, such as fighting. Destruction of the DA system causes animals to lose their desire to eat and also decreases their aversive drives (Guyton, 1981).

DA may be involved in the pathogenesis of schizophrenia (Guyton, 1981). When the nigrostriatal tract malfunctions or degenerates, the condition known as Parkinsonism results. This disease is characterized by shaking, tremor, muscular rigidity, abnormal facial movements, and difficulties in walking, speaking, and writing. The basal ganglia, the portion of the brain which encompasses most of the dopaminergic tracts, regulates, among other things, the tone and posture of the limbs (Guroff, 1980). In Parkinsonism the DA content of the caudate nucleus is about 50% of normal (Ganong, 1983). Treatment of the disease with L-dopa releases DA, alleviating motor dysfunction while sometimes producing schizophrenic symptoms, indicating that excess dopaminergic activity can cause dissociation of a person's drives and thought patterns. Certain tranquilizers that depress the DA system are commonly used in the treatment of many schizophrenic patients (Guyton, 1981).

2.5.6 THE SEROTONIN SYSTEM

The metabolism and function of serotonin (HT) have been areas of intense interest. Serotonin is formed by the conversion of tryptophan and is localized in the cell bodies in the nuclei of the lower midbrain and the upper pons called the raphe nuclei (Guroff, 1980). The axons go to all parts of the brain through the median forebrain bundle. Most of the terminals lie in the hypothalamus and very few in the cerebellum and cortex. The nerve fibers of this system spread downward into the spinal cord where they reduce the input level of pain signals (Guyton, 1981). The fibers also spread to the reticular formation and act to suppress the activity of the reticular activating system .

Drugs which reduce HT levels in the brain by 90 or 95% relate HT and sleep patterns. Serotonin neurons discharge rapidly in the awake state and slowly during sleep. Serotonin is thought to play a role in the control of mood. Serotonin has also been reported to be involved in aggression, sexual behavior, alcoholism, learning and retardation, and drug addiction (Guroff, 1980).

2.6 SUMMARY

In summary, there is a long standing history of toxicosis associated with the consumption of moldy grain. The toxic metabolites of these mold species are a major concern of the producers and consumers. The trichothecenes have been implicated as the agents responsible for moldy grain toxicosis as far back as 1891.

More recently the role of trichothecenes in central nervous system disorders has become a concern. Huff et al. (1981) demonstrated a relationship between DON and neural disturbances in chickens. Chi et al. (1981) observed alterations in brain catecholamines in chickens after orally administering T-2 toxin. The exact relationship between changes in brain catecholamines and neurosymptoms of DON and T-2 toxicosis is complicated because of the many variables that affect brain amine metabolism.

The proposed research is designed to investigate the effects of the trichothecenes DON and T-2 toxin on brain catecholamines in the whole brain and selected brain regions of rats and chickens.

Chapter III
MATERIALS AND METHODS

3.1 EXPERIMENTAL DESIGN

This study consisted of two distinct phases (Table 6). In phase I, the effects of DON and T-2 toxin on whole brain concentrations of monoamine transmitters and metabolites were determined. In phase II, the CNS effects of DON and T-2 toxin on concentrations of biogenic monoamines were determined in five distinct brain regions. Both phases of this study were performed on rats and chicks.

The toxins, deoxynivalenol (Research Foods Ltd., Downsview Ont.) and T-2 toxin (Myco-Lab Co., Chesterfield MO, USA), were dissolved into 50% ethanol and intubated into the stomach using a plastic feeding tube attached to a syringe at a dose equivalent to $2.5 \text{ mg toxin kg}^{-1}\text{bw}^{-1}$. The control group received the same amount of vehicle solution as the toxin-treated animals to compensate for the effect of ethanol on brain neurotransmitters.

3.1.1 ANIMALS

3.1.1.1 RATS

Sprague-Dawley male rats weighing 125-150 g were obtained from the University of Manitoba breeding facility. All animals were housed separately in galvanized steel cages and kept on a 14-10 hour light dark cycle. The room temperature was maintained at 21°C with a relative

humidity of 50%. Animals were fed a semi-purified diet and water ad libitum and weighed twice weekly until they reached a mean weight of approximately 180 g.

The semi-purified rat diet was formulated according to the National Research Council's guidelines set forth in Nutrient Requirements for Laboratory Animals (1978). The diet was prepared in 20 kg lots and stored at -26°C . The specific diet composition is listed in Table 4. See Appendix A for a detailed list of suppliers.

TABLE 4
Rat Diet Formulation

Casein	17.24%
DL-Methionine	0.30
Choline bitartrate	0.20
Corn starch	31.38
Glucose	31.38
Cellulose	5.00
Corn oil	5.00
Lard	5.00
AIN Mineral mix	3.50
AIN Vitamin mix	1.00
Energy	18.9 kJ/g

3.1.1.2 POULTRY

One-day-old Single Comb White Leghorn Cockerels (SCWLC) were obtained from the University of Manitoba breeding facility and were housed in electrically heated battery brooders with raised wire mesh floors.

Animals were fed a stock diet and water ad libitum until they reached 28 days of age, at which time, healthy chicks of uniform body weights were randomly distributed into one of the treatments.

A commercial chick diet, Feed-Rite Chick Starter (Feed-Rite Ltd., Winnipeg, MB), was fed to the cockerels.

TABLE 5

Chick Starter Diet, Minimum Analysis

Crude Protein	20.00%
Crude Fat	2.00
Crude Fibre	5.00
Salt	0.39
Sodium	0.15
Calcium	1.20
Phosphorus	0.75
Vitamin A	8,820 iu/kg
Vitamin D ₃	2,700 iu/kg
Vitamin E	20.00 iu/kg

3.1.2 WHOLE BRAIN STUDY

Once the rats reached a mean weight of 180 g and the chicks reached 28 days of age, they were randomly distributed into the treatment groups, and then into the sampling times. All animals were dosed at 0900 hrs. Whole brains were collected at 2, 6, 12, 24, and 48 hours after dosing. Each sampling time included 6 animals for control and each toxin-treated group.

3.1.3 BRAIN REGIONS STUDY

Similar to the whole brain study, once the rats reached a mean weight of 180 g and the chicks reached 28 days of age, they were distributed into treatment groups. Since samples were to be examined after 24 hours only, no further randomization was necessary. The brain regions selected were pons and medulla oblongata, cerebellum, hypothalamus, hippocampus, and cerebral cortex. All animals were dosed at 0900 hrs and samples were collected the following day. There were 6 animals for control and each toxin-treated group.

TABLE 6
Experimental Design

Study	hr	Control	DON	T-2 toxin
Whole Brain	2	6	6	6
	6	6	6	6
	12	6	6	6
	24	6	6	6
	48	6	6	6
Brain Regions				
pons+medulla obl.	24	6	6	6
cerebellum	24	6	6	6
hypothalamus	24	6	6	6
hippocampus	24	6	6	6
cerebral cortex	24	6	6	6

3.1.4 TISSUE DISSECTION AND PREPARATION

The animals for the whole brain study were killed by decapitation; the brains were immediately removed, frozen in liquid nitrogen, and stored at -60°C . Just prior to analysis by high performance liquid chromatography, the brains were homogenized in 10 volumes of 0.1 N perchlorate buffer and centrifuged (Beckman L5-50B ultracentrifuge, rotor type 40.3) at 4°C and 65,000 xg for 30 minutes. After centrifugation the supernatant was diluted to a final concentration of 50 mg ml^{-1} of tissue (Kotake et al., 1985).

The animals for the selected brain region experiment were killed by decapitation; the brains were immediately removed, frozen on dry ice, and then stored at -60°C until dissection. The various brain regions were dissected according to the method of Glowinski and Iversen (1966). The frozen brain to be dissected was placed on dry ice and frontal slices were cut free hand with a scalpel. The slices were kept frozen and dissected. The tissue samples were homogenized with a micropestle in 1.5 ml centrifuge tubes in the same manner as the whole brain.

3.2 HPLC METHODS

3.2.1 REAGENTS

Catechol standards were obtained from Sigma Chemical Co. (St. Louis, MI, USA). Solvents were HPLC grade; other chemicals were reagent grade; and all were obtained from Fisher Scientific (Ottawa, Canada).

3.2.2 CHROMATOGRAPHIC SYSTEM

High-performance liquid chromatography was performed using a Beckman Model 114M Solvent Delivery Module (Beckman, Toronto, Canada) liquid chromatograph. The analytical column was an Ultrasphere IP, C₁₈ column (250 x 4.6 mm ID, 5 μ m particle size) (Beckman, Toronto, Canada). Electrochemical detection was accomplished using an ESA Coulometric detector, model 5100A (Bedford, MA, USA). The catechols were oxidized at an applied potential of +0.45 V using a porous graphite electrode. Signals from the detector were recorded and integrated using a Shimadzu C-R3A Chromatopac integrating recorder.

The mobile phase was a modification of the buffer used by Martin et al. (1983) consisting of 75 mM sodium phosphate, 1.0 mM octane sulphate as an ion-pair reagent, 50 μ M EDTA and 11.5% acetonitrile. The buffer was adjusted to a final pH of 3.25. The flow rate was maintained at 1.0 ml min⁻¹.

3.3 STATISTICAL ANALYSIS

Data was analyzed using the 1983 edition of the Statistical Analysis System (SAS). Means were calculated on the control and each treatment group and were compared using a paired T-test (Steel and Torrie, 1980).

Chapter IV

RESULTS

4.1 DEOXYNIVALENOL RESULTS

4.1.1 EFFECTS OF DON ON WHOLE AND REGIONAL BRAIN CONCENTRATIONS OF MONOAMINE NEUROTRANSMITTERS AND METABOLITES IN RATS

DON did not affect the whole brain concentrations of monoamines and their metabolites in rats (Table 7). For NE the values of the control and DON treated groups were very similar. The greatest difference, 48 ng g⁻¹ tissue, was seen at 24 hours, where the concentration of NE in the DON-treated group was reduced. It was not shown to be a significant reduction, perhaps because of the large variation observed in the control and treatment groups. At 48 hours the DON treated group had NE concentrations 10% higher (41 ng g⁻¹ tissue) than the controls.

The concentration of DA for the control and DON-treated groups was also very similar. The greatest difference was observed 24 hours post dosing, where the concentration for the DON-treated group was 5% higher than the control group and remained as such until 48 hours. No significant differences between controls and treated animals were found at any sampling period. The values for DOPAC (3,4 dihydroxyphenyl acetic acid), a metabolite of dopamine, were also very similar between the control and treatment groups. The greatest difference was noted at 6 hours post dosing, at which the DON treated group was 6% higher. At both 24 and 48 hours DOPAC was similar in the control and DON treated groups.

Table 7
Effects of DON on whole brain concentrations of monoamine transmitters and metabolites in rats

Time (hrs)	Treatment	NE	DA	DOPAC	HIAA	HT
2	Control	419 ± 105	1459 ± 454	185 ± 58	317 ± 82	1568 ± 283
	DON	427 ± 102	1452 ± 251	189 ± 48	307 ± 47	1519 ± 186
6	Control	451 ± 82	1505 ± 267	198 ± 57	341 ± 92	2108 ± 1714
	DON	446 ± 148	1513 ± 379	209 ± 50	320 ± 58	2490 ± 1700
12	Control	369 ± 50	1276 ± 118	178 ± 31	246 ± 24	1982 ± 1185
	DON	379 ± 28	1283 ± 92	173 ± 22	270 ± 22	2252 ± 1162
24	Control	411 ± 85	1265 ± 192	158 ± 43	307 ± 50	1977 ± 1789
	DON	363 ± 50	1327 ± 272	161 ± 42	282 ± 45	2924 ± 1665
48	Control	365 ± 27	1341 ± 99	185 ± 23	304 ± 24	1268 ± 300
	DON	406 ± 79	1387 ± 60	192 ± 26	281 ± 19	1157 ± 227

Each value is expressed in ng/g wet tissue and represents the mean ± S.D. of 6 animals.
N.D.: Not detectable.

Table 8

Effects of DON on the regional concentrations of monoamine transmitters and metabolites in rats

Region	Treatment	NE	DA	DOPAC	HIAA	HT
Pons + Medulla	Control	460 ± 51	N.D.	N.D.	188 ± 33	496 ± 92
	DON	510 ± 74	N.D.	N.D.	288 ± 40*	1014 ± 121*
Cerebellum	Control	174 ± 40	N.D.	N.D.	77 ± 27	120 ± 44
	DON	197 ± 34	N.D.	N.D.	138 ± 53*	341 ± 197*
Hypothalamus	Control	1001 ± 311	473 ± 85	124 ± 38	327 ± 75	605 ± 86
	DON	1004 ± 252	741 ± 426	137 ± 70	469 ± 161	1273 ± 246*
Hippocampus	Control	263 ± 72	582 ± 453	N.D.	247 ± 28	410 ± 89
	DON	276 ± 42	346 ± 175	N.D.	388 ± 31*	835 ± 96*
Cerebral Cortex	Control	199 ± 32	605 ± 734	N.D.	156 ± 24	259 ± 40
	DON	166 ± 14*	618 ± 336	N.D.	198 ± 24*	524 ± 132*

Each value is expressed in ng/g wet tissue and represents the mean ± S.D. of 6 animals.

N.D.: Not detectable.

*Significantly different from the control, $p < 0.05$.

The concentration of HIAA (5 hydroxyindole acetic acid), an indoleamine and metabolite of serotonin in the control and treatment groups was also very similar. The greatest difference was seen at 12 hours, the DON-treated animals had HIAA values 10% higher than the controls. By 24 and 48 hours post-dosing HIAA was 9% and 8%, respectively, lower in the treatment group. The HT concentrations for both the control and DON-treated groups were extremely variable. The treatment group values were greater than the controls at 6, 12, and 24 hours. The greatest difference was noted at 24 hours post-dosing where the DON-treated group was 48% higher than the control group with respect to HT, but because of the large variation observed, was not significantly different.

DON was shown to affect the regional concentrations of monoamines and their metabolites in rats brains (Table 8). In the cerebral cortex, the DON-treated animals had significantly less NE (33 ng g^{-1} tissue or 20% less) than the control animals. Norepinephrine was observed to be elevated in all other brain regions; however, this difference was not found statistically significant.

Dopamine was not detectable in the pons and medulla oblongata or in the cerebellum. In the hypothalamus the DON group was shown to be 57% higher in DA than the controls. In the hippocampus the DON group was 68% lower in DA than the controls. However, neither effect was statistically significant. There was no discernible effect of DON treatment in the cerebral cortex. In all regions the lack of statistical significance may be due to the large standard error of the mean. Hypothalamus was the only tissue in which DOPAC was detectable. The DON-treated group displayed a concentration 10% higher than that of the control group. In the pons and medulla oblongata, cerebellum, hippocampus, and

cerebral cortex, the DON-treated animals had significantly greater HIAA than the controls. In all regions the DON-treated animals had significantly greater concentrations of HT than the controls. The increases, which ranged from 102 to 180%, were found to be significant in each region.

4.1.2 EFFECTS OF DON ON WHOLE AND REGIONAL BRAIN CONCENTRATIONS OF MONOAMINE NEUROTRANSMITTERS AND METABOLITES IN POULTRY

DON did not affect the whole brain concentrations of monoamine transmitters and their metabolites in poultry (Table 9). However, a notable trend occurred, all transmitters tested in the DON treated group showed marked aberrations at 2 hours post dosing. In NE there was a two-fold difference between the controls and the treatment group at 2 hours. It was not determined to be statistically significant due to a large variation. By 6 hours the treated animals had 7% less NE than the controls. At 24 hours the treatment group showed NE concentrations 15% above the controls.

Dopamine also showed a large difference between the control and DON treated groups at 2 hours. By 6 hours the effects of DON were no longer apparent; the concentration of DA for the treatment group was similar to that of the controls. At 24 hours the treatment group was showing DA concentrations 28% greater than that of the control group. The effects of DON were no longer visible at 48 hours.

There was a three-fold difference in HIAA between the DON-treated group and control animals at 2 hours, after which HIAA values in the treatment group fell to 38% those of the control group. A second peak occurred at 24 hours in the treatment group, and by 48 hours the effects,

Table 9

Effects of DON on whole brain concentrations of monoamine transmitters and metabolites in poultry

Time (hrs)	Treatment	NE	DA	HIAA	HT
2	Control	739 ± 343	821 ± 314	70 ± 26	2200 ± 850
	DON	1425 ± 768	1235 ± 400	203 ± 272	3056 ± 934
6	Control	868 ± 283	906 ± 215	N.D.	3208 ± 814
	DON	808 ± 174	959 ± 183	N.D.	3285 ± 567
12	Control	540 ± 100	673 ± 128	195 ± 280	2120 ± 469
	DON	555 ± 116	603 ± 143	74 ± 9	2026 ± 444
24	Control	524 ± 82	692 ± 118	98 ± 23	2057 ± 369
	DON	600 ± 63	886 ± 90	85 ± 10	2437 ± 281
48	Control	549 ± 45	683 ± 81	61 ± 8	2064 ± 294
	DON	492 ± 80	672 ± 47	63 ± 20	1875 ± 222

Each value is expressed in ng/g wet tissue and represents the mean ± S.D. of 6 animals.
N.D.: Not detectable.

Table 10

Effects of DON on the regional concentrations of monoamine transmitters and metabolites in poultry

Region	Treatment	NE	EPI	DA	DOPAC	HIAA	HT
Pons + Medulla	Control	1041 ± 156	111 ± 30	363 ± 32	N.D.	94 ± 21	2331 ± 302
	DON	1032 ± 115	127 ± 42	285 ± 54*	N.D.	102 ± 22	1914 ± 720
Cerebellum	Control	520 ± 48	N.D.	N.D.	N.D.	N.D.	260 ± 39
	DON	562 ± 70	N.D.	N.D.	N.D.	N.D.	254 ± 146
Hypothalamus	Control	1132 ± 220	147 ± 53	3737 ± 1267	147 ± 44	126 ± 42	3059 ± 368
	DON	817 ± 225*	89 ± 56	4170 ± 792	152 ± 58	102 ± 31	2575 ± 897
Hippocampus	Control	372 ± 113	N.D.	1758 ± 568	N.D.	74 ± 5	2473 ± 448
	DON	233 ± 75*	N.D.	912 ± 531	N.D.	59 ± 21	1716 ± 794
Cerebral Cortex	Control	306 ± 20	N.D.	128 ± 24	N.D.	N.D.	1559 ± 129
	DON	305 ± 39	N.D.	85 ± 20	N.D.	N.D.	1353 ± 411

Each value is expressed in ng/g wet tissue and represents the mean ± S.D. of 6 animals.

N.D.: Not detectable.

*Significantly different from the control, $p < 0.05$.

of DON appear to have subsided. Serotonin also showed an initial increase at 2 hours and again at 24 hours. The greatest difference occurred at 2 hours post dosing where the treatment group was 40% higher than the control.

DON was responsible for a few significant changes in regional concentrations of monoamine transmitters and their metabolites in poultry (Table 10). The hypothalamus and hippocampus in the treatment group showed significantly lower concentrations of NE (72% and 63%, respectively). NE values in the pons and medulla oblongata, cerebellum and the cerebral cortex for both groups were similar. The pons and medulla oblongata and hypothalamus of the chick also contained measurable amounts of EPI. The treatment group was shown to have 40% less hypothalamic EPI than the control group, yet the pons and medulla of the treatment group reported a slightly greater concentration (14%) of EPI compared to the controls.

Lower concentrations of DA were reported in the pons and medulla oblongata, hippocampus, and cerebral cortex of the treatment group. The results were significant in the pons and medulla oblongata. The hypothalamus was found to have less DA in the control group, a large variation was observed. DOPAC, a metabolite of DA, was found to be lower in the control hypothalamus also.

Both HT and HIAA were lower in the DON treated groups in most regions. No significant differences were found but it is believed to be an important trend.

4.2 T-2 TOXIN RESULTS

4.2.1 EFFECTS OF T-2 TOXIN ON WHOLE AND REGIONAL BRAIN CONCENTRATIONS OF MONOAMINES NEUROTRANSMITTERS AND METABOLITES IN RATS

T-2 toxin, like DON did not effect the whole brain concentrations of monoamine transmitters and metabolites in rats (Table 11). An interesting trend occurred at 2 hours post-dosing. In T-2 toxin-dosed rats, NE, DA, DOPAC, HIAA, and HT all showed marked differences from the control. The level of NE in the treatment group was 5.4% greater than that of the control at 2 hours. Other than the apparent differences between the two groups at 2 hours, the NE profile of the treatment group resembled that of the control. Levels of the treatment group remained suppressed until 24 hours (12%, 7%, and 9% lower than the control group at 6, 12, and 24 hours). By 48 hours levels for the two groups were indistinguishable. It would seem as though T-2 toxin manifests itself maximally in the rat at 2 hours post dosing and is metabolized by 48 hours.

A similar occurrence may be seen in DA and DOPAC. An initial increase (9% in DA and 10% in DOPAC) in the treatment group followed by a suppression at 6, 12, and 24 hours (8% and 1% in DA and 12% and 3% in DOPAC at 12 and 24 hours, respectively). The greatest difference between the control and T-2 toxin-treated groups was observed at 12 hours for both DA and DOPAC. By 48 hours the effects of the T-2 toxin were less apparent.

HIAA was 23% higher in the treatment group than in the controls at 2 hours. The concentration declined steadily until 24 hours at which point it was 12% less than the control. By 48 hours the difference represented only a 5% reduction in the treatment group. HT was 10%

Table 11

Effects of T-2 toxin on the whole brain concentrations of monoamine transmitters and metabolites in rats

Time (hrs)	Treatment	NE	DA	DOPAC	HIAA	HT
2	Control	419 ± 105	1459 ± 454	185 ± 58	317 ± 82	1568 ± 283
	T-2 toxin	442 ± 75	1592 ± 502	204 ± 73	390 ± 83	1721 ± 210
6	Control	451 ± 82	1505 ± 267	198 ± 57	341 ± 92	2108 ± 1714
	T-2 toxin	395 ± 65	1451 ± 210	205 ± 34	331 ± 36	2360 ± 1346
12	Control	369 ± 50	1276 ± 118	178 ± 31	246 ± 24	1982 ± 1185
	T-2 toxin	342 ± 63	1177 ± 240	157 ± 31	288 ± 59	2665 ± 949
24	Control	411 ± 85	1265 ± 192	158 ± 43	307 ± 50	1977 ± 1789
	T-2 toxin	373 ± 112	1248 ± 225	153 ± 34	269 ± 55	2261 ± 1798
48	Control	365 ± 27	1341 ± 99	185 ± 23	304 ± 24	1268 ± 30
	T-2 toxin	374 ± 32	1424 ± 30	170 ± 21	290 ± 24	1159 ± 28

Each value is expressed in ng/g wet tissue and represents the mean ± S.D. of 6 animals.

N.D.: Not detectable.

Table 12

Effects of T-2 toxin on the regional concentrations of monoamine transmitters and metabolites in rats

Region	Treatment	NE	DA	DOPAC	HIAA	HT
Pons + Medulla	Control	460 ± 51	N.D.	N.D.	188 ± 33	496 ± 92
	T-2 toxin	606 ± 77*	N.D.	N.D.	435 ± 54*	1256 ± 192*
Cerebellum	Control	174 ± 40	N.D.	N.D.	77 ± 27	120 ± 44
	T-2 toxin	220 ± 47	N.D.	N.D.	139 ± 37*	275 ± 91*
Hypothalamus	Control	1001 ± 311	473 ± 85	124 ± 38	327 ± 75	605 ± 86
	T-2 toxin	846 ± 288	786 ± 438	199 ± 76	568 ± 85*	1496 ± 222*
Hippocampus	Control	263 ± 72	582 ± 453	N.D.	247 ± 28	410 ± 89
	T-2 toxin	324 ± 80	433 ± 447	N.D.	536 ± 143*	1043 ± 379*
Cerebral Cortex	Control	199 ± 32	605 ± 734	N.D.	156 ± 24	259 ± 40
	T-2 toxin	201 ± 39	223 ± 248	N.D.	276 ± 96*	815 ± 472*

Each value is expressed in ng/g wet tissue and represents the mean ± S.D. of 6 animals.

N.D.: Not detectable.

*Significantly different from the control, $p < 0.05$.

higher in the treatment group at 2 hours and by 12 hours was 34% higher than the control. The difference between the two groups decreased at 24 hours and by 48 hours; the T-2 toxin-treated group had HT levels 9% less than the controls.

T-2 toxin was responsible for several alterations in regional concentrations of the monoamines and their metabolites in rats (Table 12). Differences could be seen in the NE levels in the pons and medulla oblongata, cerebellum, hypothalamus, and hippocampus. NE was higher in the T-2 toxin-treated group in all regions but the hypothalamus where the control group had greater NE values (18%). These differences were significant in the pons and medulla oblongata only.

Measurable amounts of DA were found in the hypothalamus, hippocampus and cerebral cortex of the rat. All regions showed differences between the two groups. The hypothalamus showed higher levels of DA in the treatment group (66%). Both the hippocampus and cerebral cortex had lower DA levels in the treatment group (26% and 63% lower); however, these differences were not statistically significant because of the large variability. DOPAC was found in the hypothalamus only and, like DA, the difference observed was not significant.

The administration of T-2 toxin caused changes in both HIAA and HT in the pons and medulla oblongata, cerebellum, hypothalamus, hippocampus and cerebral cortex. The T-2 toxin-treated group were found to have significantly higher levels of both HIAA and HT in all regions.

4.2.2 EFFECTS OF T-2 TOXIN ON WHOLE AND REGIONAL BRAIN CONCENTRATIONS OF MONOAMINE NEUROTRANSMITTERS AND METABOLITES IN POULTRY

No significant differences were found in poultry whole brains between the control and T-2 toxin treated groups at any time (Table 13). For NE the value of the control and T-2 toxin-treated groups were very similar. Slight differences were observed at 24 hours where the T-2 toxin treated group was 14% higher than the control. At 48 hours the treated group had NE levels significantly lower than those of the control group.

Dopamine showed a 7% difference between the two groups at 6 hours. At 24 hours the T-2 toxin-treated group was 32% greater than the control. This difference was transient with DA concentrations of the two groups only slightly different (5%) at 48 hours. None of the differences were statistically significant.

A difference of 30% was found between HIAA values of the control and treatment groups at 2 hours. It appears that the brain concentration of HIAA fell below a detectable level at 6 hours after the initial peak at 2 hours. The greatest difference was seen at 12 hours when the control group peaked and the treatment group was but 44% of the control group. The treatment group peaked at 24 hours and was 33% higher than the controls. No differences were found between the control and T-2 toxin treated groups at 48 hours. The values of the control and treatment groups for HT were very similar until 24 hours. A difference of 21% was found when HT of the T-2 toxin-treated group peaked at 24 hours. By 48 hours the treatment group had HT values similar to that of the controls.

Poultry appeared to be less susceptible than rats to the effects of T-2 toxin (Table 14). Three T-2 toxin-treated rats died between 24 and

Table 13

Effects of T-2 toxin on whole brain concentrations of monoamine transmitters and metabolites in poultry

Time (hrs)	Treatment	NE	DA	HIAA	HT
2	Control	739 ± 343	821 ± 314	70 ± 26	2200 ± 850
	T-2 toxin	797 ± 487	815 ± 223	91 ± 24	2109 ± 657
6	Control	868 ± 283	906 ± 215	N.D.	3208 ± 814
	T-2 toxin	835 ± 204	969 ± 221	N.D.	3296 ± 747
12	Control	540 ± 100	673 ± 128	195 ± 280	2120 ± 469
	T-2 toxin	551 ± 60	703 ± 103	86 ± 17	2080 ± 318
24	Control	524 ± 82	692 ± 118	98 ± 23	2057 ± 369
	T-2 toxin	589 ± 259	910 ± 401	131 ± 90	2491 ± 1069
48	Control	549 ± 45	683 ± 81	61 ± 8	2064 ± 294
	T-2 toxin	477 ± 43*	721 ± 52	62 ± 13	1881 ± 173

Each value is expressed in ng/g wet tissue and represents the mean ± S.D. of 6 animals.
N.D.: Not detectable.

Table 14

Effects of T-2 toxin on the regional concentrations of monoamine transmitters and metabolites in poultry

Region	Treatment	NE	EPI	DA	DOPAC	HIAA	HT
Pons + Medulla	Control	1041 ± 156	111 ± 30	363 ± 32	N.D.	94 ± 21	2331 ± 302
	T-2 toxin	817 ± 228	101 ± 7	225 ± 80*	N.D.	184 ± 80*	2229 ± 788
Cerebellum	Control	520 ± 48	N.D.	N.D.	N.D.	N.D.	260 ± 39
	T-2 toxin	415 ± 99*	N.D.	N.D.	N.D.	N.D.	197 ± 69
Hypothalamus	Control	1132 ± 220	147 ± 53	3737 ± 1267	147 ± 44	126 ± 42	3059 ± 368
	T-2 toxin	1091 ± 199	139 ± 34	3432 ± 1205	242 ± 95	211 ± 65*	3314 ± 661
Hippocampus	Control	372 ± 113	N.D.	1758 ± 568	N.D.	74 ± 5	2473 ± 448
	T-2 toxin	273 ± 92	N.D.	1056 ± 428	N.D.	131 ± 64	2450 ± 689
Cerebral Cortex	Control	306 ± 20	N.D.	128 ± 24	N.D.	N.D.	1559 ± 129
	T-2 toxin	169 ± 34*	N.D.	105 ± 48	N.D.	N.D.	1411 ± 332

Each value is expressed in ng/g wet tissue and represents the mean ± S.D. of 6 animals.

N.D.: Not detectable.

*Significantly different from the control, $p < 0.05$.

48 hours post-dosing and several rats showed signs of neurological disturbances.

There were a few noticeable effects of T-2 toxin on regional transmitter concentrations in poultry. The treatment group had NE levels lower than those of the controls in all regions. The differences were statistically significant in the cerebellum and cerebral cortex (20% and 45% lower, respectively). Epinephrine was measureable in the pons and medulla oblongata and hypothalamus. Slightly lower values were found for the treatment group (10% and 5% lower, respectively). These differences were not significant.

Lower DA values were reported for the treatment group in the pons and medulla oblongata, hypothalamus, hippocampus, and cerebral cortex. A significant difference (38% lower) was found in the pons and medulla oblongata. DOPAC was found in the hypothalamus only, in which case lower values were reported in the control group. This may be explained by the fact that the hypothalamus displayed only an 8% difference between the two groups and showed a large variation in DA (35% in the treatment group).

HIAA was found to be consistently higher in the treatment group than control group in the pons and medulla oblongata, hypothalamus, and hippocampus (96%, 67%, and 77%, respectively). The difference was significant in the pons and medulla oblongata and hypothalamus. The differences found with HT were quite variable. Lower levels were shown for the treatment group in the cerebellum and cerebral cortex (24% and 9%, respectively), but in the hypothalamus the values for the treatment group were found to be 8% higher than those of the control group. The

pons and medulla oblongata and hippocampus showed no differences between the two treatments.

Chapter V

DISCUSSION

5.1 DEOXYNIVALENOL STUDY

In the present study, rats and chicks were dosed with 2.5 mg DON kg⁻¹bw⁻¹. No deaths occurred in either species, nor were any clinical signs of DON toxicosis observed. Slight loss of appetite was observed in rats, but that was also seen in the control group, and was probably a result of the vehicle solution. Outward appearances of the animals were normal. In the chicks alertness, coordination, and feathering of the two groups were comparable. para;DON caused no significant changes in whole brain concentrations of neurotransmitters in either rats or chicks. The differences between the treatment group and the controls for both species were very erratic. There does not appear to be any trend with respect to an elevation or a suppression of any of the monoamines in rats. However, in poultry a trend was apparent at 2 hours. Markedly higher values of NE, DA, HIAA, and HT were seen in the treatment group compared to the control group. By 48 hours the effects of DON appeared to have subsided.

DON was responsible for several alterations in the regional concentrations of monoamines and their metabolites in rats and poultry. HIAA and HT, the indoleamines, were significantly higher in the DON treated rats in all regions. This is an important observation, and may indicate an apparent alteration in the metabolism of the indolamines. The

regional brain concentrations of the catecholamines were unaltered, except in the cerebral cortex, NE of the DON-treated animals was significantly reduced.

Chickens are reportedly more resistant to the effects of DON. The LD₅₀ value for a single oral dose in mice is 46 mg DON kg⁻¹bw⁻¹ (Yoshizawa and Morooka, 1977), and 140 mg DON kg⁻¹bw⁻¹ in poultry (Huff et al., 1981). Swine are sensitive to the effects of DON. Trenholm et al. (1984) found that pigs can eat diets containing up to 2 ppm DON without serious harm to health or productivity. Poultry have been shown to consume up to 5 ppm DON without deleterious effects. In the present study, DON did not appear to have any affect on the indoleamines, HIAA and HT, of poultry. However, NE was significantly reduced in the hypothalamus and hippocampus of the treated animals, and DA was reduced in the pons and medulla oblongata of the treatment group.

5.2 T-2 TOXIN STUDY

T-2 toxin is a potent mycotoxin. The LD₅₀ value for a single dose of T-2 toxin in broiler cockerels is reported to be between 4.0 mg T-2 toxin kg⁻¹ bw⁻¹ (Hoerr et al., 1981), and 5.25 mg kg⁻¹ bw⁻¹ (Chi et al., 1977a). However, the administration of 2.5 mg T-2 toxin kg⁻¹ bw⁻¹ in this study did not result in the deaths of any cockerels at any sampling time. Gross examination did not reveal any lesions that could be attributed to T-2 toxin. No clinical signs of T-2 toxicosis were evident in any chickens other than slightly fluid fecal material.

Rats were more susceptible to T-2 intoxication than were poultry. The T-2 toxin treated animals showed signs of tremors, inappetence, and

listlessness. No deaths were reported in any of the animals sacrificed 2, 6, 12 or 24 hours post dosing. Of the 7 rats that received 2.5 mg kg^{-1} T-2 toxin in the 48 hour sampling group, 3 died 36 to 48 hours post-administration of the toxin. The LD_{50} value for a single oral dose of T-2 toxin in male rats is 4.0 mg T-2 toxin kg^{-1} bw^{-1} (Marasas et al., 1969).

This study found no measurable effects of T-2 toxin in whole brain concentrations of neurotransmitters in either rats or chickens. A sharp increase in all monoamines and their metabolites was seen at 2 hours in the whole brains of rats. A very rapid change in NE, DA, DOPAC, HIAA, and HT occurred as a result of exposure to T-2 toxin. Poultry are reportedly more tolerant to the effects of T-2 toxin than swine and rats (Hoerr et al., 1981). For all, NE, DA, HIAA and HT, the values of the control and T-2 treated groups were very similar. However, at 24 hours the treatment group was reporting values 14%, 32%, 33%, and 21% higher than the control group for NE, DA, HIAA, and HT, respectively.

As was expected, the effects of the toxins were more evident in the specific brain regions as compared to whole brain. T-2 toxin was responsible for several alterations in the regional concentrations of neurotransmitters in both rats and chickens. In rats, T-2 toxin-treated animals showed changes similar to those of the DON-treated animals. Values for HT and HIAA were significantly higher in the treatment group in all regions. NE was the only catecholamine found to be significantly altered, it was elevated in the pons and medulla oblongata of the treatment group.

In poultry, the treatment group reported significantly lower NE in the cerebellum and cerebral cortex, and lower DA in the pons and medulla oblongata. Also, HIAA was significantly higher in the treatment group in the pons and medulla oblongata and hypothalamus.

Mortality in acute trichothecene intoxication is thought to be caused by cardiorespiratory failure (Ueno, 1977). Feuerstein et al. (1985) stated that a triphasic circulatory response is caused by intravenous infusion of T-2 toxin to rats. An initial decrease in heart rate and blood pressure is followed by tachycardia and hypertension, which may lead to death due to cardiovascular and respiratory failure.

Siren and Feuerstein (1986) observed marked impairment of blood flow and increased vascular resistance in hindlimb skeletal muscles, mesenteric and renal vascular beds. The detrimental action of T-2 toxin after acute parenteral administration appears to impair blood flow in vital organs as a result of reduced cardiac output and peripheral vasoconstriction. This increase in vascular restriction became apparent after one hour and reached its maximum in 4 to 6 hours. T-2 toxin has recently been shown to produce vasoconstriction in a bovine ear perfusion system (Wilson and Gentry, 1985).

Lesions in myocardial capillaries were observed within one hour of initiation of a perfusion of T-2 toxin in a rat heart (Yarom and Yagen, 1986). The lesions were characterized by dilation of the microvessels, swelling of the microvessels, swelling of the endothelial cells, damage to the plasma membrane followed by tearing of vessel walls and extravasation of erythrocytes. It was thought that damage to the capillaries may have been produced through mediation of the central nervous system.

T-2 toxin alters the respiratory and blood pressure centres causing a hypotensive shock-like state. Dilation of the microvasculature does occur in shock, but such lesions do not normally occur. The authors state that changes in brain prostaglandin synthesis are probably a direct result of T-2 toxin on the brain but that the hemodynamic responses to T-2 toxin at the same time is unclear. Feuerstein et al. (1985) have reported that T-2 toxin administration caused an increase in plasma catecholamines in the rat and guinea pig, suggesting that T-2 toxin induced impaired cardiac output and increased vascular resistance is mediated by the action of the sympathetic nervous system, thus implying a role of the vascular smooth muscle on increased vascular resistance.

While it seems possible that T-2 toxin alters the microvasculature through its effects on the CNS, the study by Wilson and Gentry (1985) offers conflicting information. They reported vasoconstriction using an in vitro bovine ear perfusion system, thus indicating that T-2 toxin directly affects the bovine vasculature. The authors speculated that T-2 toxin may interact with nonspecific cellular receptors on bovine blood vessels. Yarom et al. (1984) demonstrated in their study in platelets that T-2 toxin can interact with cell membranes and induce changes in cell membrane. At this time, changes in brain neurotransmitters appear to be a result of both the peripheral and central mechanisms of T-2 toxin.

Chapter VI

CONCLUSION

Oral administration of DON and T-2 toxin did not influence whole brain concentrations of monoamines or their metabolites in either rats or chicks at any time. However, alterations in the regional concentrations of monoamine transmitters and metabolites were seen in both species.

In general, two phenomena were observed; 1) an effect of the toxins on the indoleamines in rats and 2) an effect of the toxins on the catecholamines in chicks. In rats, the administration of both DON and T-2 toxin resulted in significantly higher concentrations of HIAA and HT in all regions. A few other differences were noted. Significantly lower NE was seen in the cerebral cortex of DON-treated rats, and a significantly greater concentration of NE in the pons and medulla oblongata in the T-2 toxin-treated rats. In poultry, the administration of DON and T-2 toxin had a profound effect on the catecholamines. NE concentrations were found to be significantly less in the hypothalamus and hippocampus of DON-treated chicks and T-2 toxin-treated chicks were found to have significantly less NE in the cerebellum and cerebral cortex. DA was significantly less in the pons and medulla oblongata of both the DON- and T-2 toxin-treated chicks.

The results of this study appear to indicate that DON and T-2 toxin have deleterious effects on the central nervous system. These results also suggest that there is an intraspecies difference in the central effects of DON and T-2 toxin.

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Appendix A

LIST OF NUTRIENT SUPPLIERS

CARBOHYDRATES

Corn Starch	Casco Brand, Canada Starch Co. Cardinal, Ontario
Alphacel Non-Nutritive Bulk	ICN Pharmaceuticals Inc. Life Sciences Group 26201 Mills Road Cleveland, Ohio
Dextrose	R. Wine Baril Winnipeg, Manitoba

PROTEIN

Vita-Free Casein	United States Biochemicals 21000 Miles Parkway Cleveland, Ohio
DL-Methionine	United States Biochemicals

Fat

Mazola Corn Oil	Best Foods PO Box 129, Sta. A Montreal, Quebec
Tenderflake Lard	Maple Leaf Foods Canada Packers

VITAMINS AND MINERALS

AIN VITAMIN Mixture 76	ICN Pharmaceuticals
AIN Mineral Mixture 76	ICN Pharmaceuticals
Choline Bitartrate	United States Biochemicals