CLINICAL SIGNS AND HISTOPATHOLOGIC CHANGES OF THE SPINAL CORD IN PIGS TREATED WITH TRI-O-CRESYL PHOSPHATE

by

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TABLE OF CONTENTS

INTRODUCTION

The organophosphate tri-o-cresyl phosphate (TOCP) is known to produce delayed neurotoxicity in man and animals. Cases of accidental human exposure to TOCP and subsequent development of neuropathy are well documented.

Smith and his colleagues (cited by Cavanagh, 1964) showed that an oral dose of .5 ml/kg (500 mg/kg) of TOCP would produce paralysis in chickens. To date, the hen has served as the main animal model for the testing of new organophosphate compounds before they are brought on the market. TOCP, at the 500 mg/kg level, is frequently employed to provide a positive control.

Kruckenberg, et al (1973) demonstrated that pigs exposed to multiple topical applications of TOCP would develop posterior paresis. Other reports of organophosphate-induced delayed neurotoxicity are present in the literature. Based on this knowledge it was felt that the pig might serve as a suitable alternative to the hen for new drug testing.

The specific objectives of the study were: (1) to determine whether the pig is clinically as sensitive as the hen to a single oral dose of TOCP at 500 mg/kg and (2) to note any histopathologic changes in the spinal cords of TOCP treated pigs.

LITERATURE REVIEW

Tri-o-cresyl phosphate (TOCP) is one of a group of organophosphorous esters that has been shown to produce delayed neurotoxicity in man and animals. It should be noted that tritolyl phosphate (TOTP) is the same as tri-o-cresyl phosphate (TOCP). Tritolyl phosphate is the preferred name, but the compound is more widely known in the medical community as tri-o-cresyl phosphate or TOCP and will be referred to as such in this paper. In recent history a number of incidents of accidental human exposure to TOCP and the subsequent development of neuropathy have been documented. These cases served to stimulate scientific investigation of the pathogenesis of organophosphate-induced delayed neurotoxicity and have furthered our understanding of the phenomenon.

In 1929-1930, several thousand people in the United States became poisoned when they drank an alcoholic extract of ginger and rum, known as "ginger-jake", which was contaminated with TOCP (cited by Hopkins, 1975). During prohibition, Extract of Ginger, U.S.P., was used as a beverage by Americans for its alcohol content. Problems came about when substitutes were used to dilute the oleoresin of ginger. Lindol, an oil used in the celluloid and varnish industry, was used in the adulteration of Extract of Ginger, since it was cheap and miscible in alcohol and with the oleoresin of ginger. Tri-o-cresyl phosphate, along with small amounts of the meta- and para-forms, comprised the major portion of Lindol. According to the U.S. Bureau of Prohibition statistics, this resulted in the paralysis of some 20,000 people (cited by Cavanagh, 1964).

Smith et al produced paralysis in chickens with an oral dose of .5 ml/kg of TOCP. Signs of paralysis began by the ninth or tenth day and eventually progressed to a point in which the chickens could no longer

stand, usually by the nineteenth or twentieth day. Weakness was seen first in the legs and then in the wings after several days (cited by Cavanagh, 1964).

Kruckenberg et al (1973) found TOCP to induce posterior paresis in swine. TOCP was poured on the dorsum of the thorax every 48 hours for a total of 83 treatments at a dosage rate of 128 mg/kg of body weight. The swine were observed daily for signs of acute toxicosis, appetite, mental alertness, and sequential development of posterior paresis. No signs of acute poisoning were manifested.

An arbitrary classification system for categorization of posterior paresis in swine was set forth by Kruckenberg et al (1973):

> "Sequential development of clinical signs of posterior paresis was classified into five groups: mild, moderate, posterior incoordination, partial posterior paresis, and total posterior paresis. Mild signs of developing posterior paresis including stationary and walking hypermetria, occasional "stringhalt" movements when walking, slight medial deviation of the toes of the rear legs when walking, and occasional knuckling-over on the fetlock joint and shifting the weight from one rear leg to the other while standing. Moderate signs included consistent and easily detected hypermetria, medial deviation of the toes of the rear legs when walking (to the point that they touched the opposite leg), frequent knuckling-over of the fetlocks (as if tripped), and falling when trying to stop or turn quickly. Posterior incoordination was a point in development of neurotoxic signs that included falling down in the rear quarters when standing. The pig could still rise by himself, but would frequently fall. Partial posterior paresis was indicated when the pig could still rise to his feet, but would fall after a few steps. 'Dog-sitting' was the most frequently seen position; assisted with a tail lift, pigs could stand and walk a few steps. Complete posterior paresis was when the pig could not rise to his feet or stand, even if assisted to his feet by a tail lift."

Mild signs of posterior paresis were seen as early as the twenty-fourth experimental day and as late as day 136. The average time to the onset of mild signs was 47 days. Total posterior paresis was seen on the average by day 100. The earliest onset of total paresis was 70 days and the longest time to onset was 166 days.

Prior to this study, it had been shown that another organophosphate, 2,2-dichlorovinyl methyl octyl phosphate, produced neurotoxicosis in swine topically exposed to the compound. Clinical signs of neurotoxicosis were found to occur from 2 weeks to several weeks after exposure, depending on the fractional or total dosage (Kruckenberg et al, unpublished data, 1969).

In recent years a number of cases of posterior paralysis in swine in England and Scotland have been reported (Stubbings et al, 1976). All of these cases occurred subsequent to worming with the organophosphorous preparation 0,0 di-(2-chloroethyl) 0-(3-chloro-4-methylcourmarin-7-yl) phosphate (Haloxon) 1 to 4 weeks previously. The main swine groups affected were sows and weanlings, and the paralysis was typically manifested approximately 3 weeks after worming. Clinical signs varied from, in the mildest cases, knuckling of the hind limbs to total paralysis of the hind limbs in the most severely affected pigs. Most of the pigs exhibited marked flexion of the metatarsal joints and would make feeble walking movements with the hind limbs. No anorexia or loss of thriftiness was seen in any of the pigs except the worst affected. Stubbings et al (1976) felt that the rate of onset of signs and the degree of paralysis attained was dose-related. No response to drug therapy was noted but under good nursing care some of the weanlings did become mobile again while retaining an abnormal gait. Others recovered partially and then relapsed, and some showed no clinical improvement (Stubbings et al, 1976).

Malone earlier had encountered delayed neurotoxicity in pigs which he attributed to Haloxon (cited by Aldridge et al, 1969). Christian, in 1975, reported on neurotoxicity in swine following an apparently normal anthelmintic dose of Haloxon (cited by Bradley, 1976).

In a recent study, Wilson et al (in press, 1982), used various single oral doses of TOCP in 6 to 9 month old Duroc-Yorkshire gilts weighing an average of 37 kg (range of 21 to 50 kg). They treated the principals with single dosages, per os, of 100, 400, 800, and 1600 mg/kg. At all dosage levels pigs exhibited acute clinical signs of mild depression and anorexia. Ataxia was seen in all groups by day 15 post-treatment. Severe posterior paresis ensued in pigs that had received the 3 highest dosage levels. Of the 5 gilts in the 100 mg/kg treated group, 1 developed slight posterior paresis, 1 developed moderate signs, and 3 progressed to severe posterior paresis.

The fundamental lesion of delayed neurotoxicity in the hen due to TOCP poisoning is believed to be a "dying back" polyneuropathy in which axonal degeneration begins at the most distal portion of the axon and proceeds toward the cell body, which may undergo chromatolysis (Beck et al, 1977). The neurotoxic lesions are seen most readily in the long sensory (ascending) and motor (descending) tracts at the distal end of the axon. Lesions in the ascending tracts are seen in the cervical region and in the descending tracts in the dorsal (thoracic) and lumbo-sacral region of the spinal cord. Lesions have been observed at all levels of the spinal cord but with a greater frequency in the upper and mid-cervical region. The lumbosacral region tends to have relatively few lesions (Bradley, 1976).

Cavanagh (1964), in experimental studies using TOCP in the hen, showed that delayed neurotoxic lesions produced in the peripheral and central nervous systems were the result of wallerian degeneration and not primary demyelination. He concluded that it was primarily the large diameter fibers of the peripheral nervous system that were damaged by the intoxication, most of the lesions occurring in the distal aspect of the longest

fibers. In the spinal cord of hens, he noted that lesions in the sensory (ascending) pathways tended to concentrate in the more cranial portion of the tracts and lessen in frequency and severity as the cell bodies in the spinal ganglia and cord were approached. Of the ascending pathways the spinocerebellar tracts in the medulla, dorsolateral in location, and cervical spinal cord were the most severely affected. In the thoracolumbar region of the spinal cord, only minimal damage to fibers in the tracts was noted.

Ventral tracts lying close to the midline in the lumbosacral region of the chicken spinal cord were the only descending tracts consistently showing lesions. Damage occurring in these tracts diminished in spinal cord cross-sections anterior to the lumbosacral level (cited by Cavanagh, 1964).

Recently, teased-fiber studies by Boulden and Cavanagh (1979) of peripheral nerves of cats treated with the organophosphate di-isopropylfluorophosphate (DFP) have challenged the traditional "dying-back" neuropathy hypothesis. They found that that the neurotoxic organophosphate induced a focal, distal but not terminal, axonal degeneration. These workers proposed that this "chemical transection" of the axon then precipitates the classic wallerian degeneration of the distal axon.

In spinal cord transverse sections with hematoxylin and eosin staining, axonal degeneration is seen as a solid eosinophilic mass, occasionally slightly basophilic and sometimes foamy and faintly eosinophilic. The swollen axon breaks up to form globules which vary in size, depending upon where the axon was sectioned (Bradley, 1976).

As degeneration of nerve fibers and myelin breakdown is occurring, there is a reactive increase of glial cells around the damaged fibers. Microglia become activated and increase in number (Cavanagh et al, 1979).

Examination of the nerve cell bodies in the spinal cord and the spinal root ganglia of the hen revealed no pathological change before and during the development of TOCP-induced paralysis (Cavanagh et al, 1979). Chromatolytic change in the spinal and motor nerve cells was sometimes seen after 3 weeks post-treatment and was attributed to be a response to the distal changes in the nerve fibers of the cells (Cavanagh, 1964).

Bickford et al (1982) studied the types and incidence of spontaneous lesions of the nervous system of healthy commercial adult hens. Tissue sections were examined for perivascular cuffing and glial cell proliferation (gliosis), axonal degeneration, neuronal swelling and chromatolysis, and vacuolization of white matter. Mild background neuropathology was noted in most of the spinal cord sections examined. The investigators pointed out that background neurologic lesions in clinically normal research animals may be the result subclinical diseases, exposure to attenuated live-virus vaccines, aging, or deficiency of certain nutrients. These residual lesions can interfere with the evaluation of morphological alterations in neurotoxicity studies.

MATERIALS AND METHODS

Twelve female Yorkshire pigs were selected. Six pigs were used as controls and 6 pigs were treated orally with a single dose of tri-o-tolyl phosphate (from mixed cresols)[1] at 500 mg of TOCP/kg.

A commercial ration (15% protein) and water were made available ad libitum. At the start of the experiment, all pigs were determined to be in good health. Ten days before the experiment was started, mean body weight for the 6 control pigs was 8.6 kg, and for the 6 principals 8.9 kg. At the time the dose was administered, the body weight of controls averaged 12.3 kg, and that of principals 12.2 kg. The pigs were housed in the Kansas State University Veterinary Clinical Science Building for the duration of the study.

Pigs were observed for acute toxicosis at .25, .5, 1, 2, 4, 8 and 16 hours following treatment and then daily thereafter for mental alertness; walking and static hypermetria; equilibrium; unsteadiness of the pelvic limbs while standing, walking, running, turning, or stopping; placement of the rear feet when standing, walking, running, turning, or stopping; knuckling-over of the fetlock joint when walking; "dog sitting" position when resting, eating, or drinking; alteration in the arc of the rear legs when walking (medial and lateral deviation of legs during locomotion included); posterior incoordination, partial paresis, and total paresis; anterior ataxia or paresis; and prolapsed rectum. Five arbitrary classifications (mild signs, moderate signs, posterior incoordination, partial posterior paresis, and total posterior paresis) were used for classification of signs of delayed neurotoxicity. (From Kruckenberg et al, 1973).

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[1] Technical grade (Lot B4A), Eastman Kodak Company, Rochester, N.Y.

At the end of the 42 day observation period, all 12 pigs were euthanatized via electrocution (110 volts).

Necropsies were conducted on all pigs. The spinal cords were fixed in 10% buffered neutral formalin, embedded in paraffin, cut 9 μm thick, and stained with hematoxylin and eosin.

Three spinal cord sections from each pig (C_4, T_7 and L_4) were evaluated using the parameters described by Bickford and Sprague (1982) - (axonal degeneration, perivascular cuffing, glial cell proliferation, neuronal swelling and chromatolysis, and vacuolization of white matter). In addition to the above parameters, the presence of hemorrhage in the gray matter was also evaluated.

RESULTS

Changes in the body weights of the control and treated groups during the experimental period are presented in Table 1. The average daily gain for the untreated pigs was 0.48 kg and 0.47 kg for the TOCP treated pigs. There were no statistically significant differences between the weights of the groups at any of the 7 weighing periods.

None of the pigs in the study developed signs of acute organophosphate toxicity. During the first 21 days post-exposure no clinical signs of delayed neurotoxicity were noted. Mild signs of delayed neurotoxicity (mild over-flexion of the hock and tendency toward medial deviation of the rear limbs) were noted in 2 of the 6 pigs on the twenty-fourth day post-treatment (Table 2). On the twenty-fifth experimental day 2 more pigs in the treated group displayed mild clinical signs. Through the remainder of the experimental period, signs in the 4 affected pigs did not advance beyond the mild stage. Two pigs from the treated group remained normal during the study.

Histopathologic examination of spinal cord cross-sections obtained in the study revealed axonal swelling and myelin sheath distension to be parameters in which the groups differed most dramatically. A comparison of the combined totals of swollen axons from the 3 spinal cord levels appears in Table 3. The treated group had a significantly greater number of swollen axons than the controls. In Table 4 the combined totals of distended myelin sheaths of the groups were compared. No significant difference in the number of distended myelin sheaths was found between the treated and control pigs when all three levels of the cord were pooled.

At the level of the fourth cervical nerve (C_4) there were significantly more swollen axons in the treated pigs (Table 3). The number of dis-

tended myelin sheaths at the C_4 level was also significantly greater in the treated group (Table 4).

Thoracic and lumbar sections from the groups yielded counts of swollen axons and distended myelin sheaths that were, with one exception, greater in the treated group. For these sections, however, neither of the parameters revealed differences between the treated and control groups large enough to be statistically significant (Tables 3 and 4).

Table 5 presents a summation of the other histopathologic lesions observed in the groups. Notable among these finding was perivascular cuffing, in which the controls had a significantly greater number of cord sections displaying the change.

DISCUSSION

Based on the findings of this study one can conclude that the pig is not as susceptible as the hen to a single dose of TOCP at 500 mg/kg. When the hen test dose of TOCP (500 mg/kg) was administered per os, the pigs developed only mild clinical signs of posterior paresis. The change was seen primarily as a subtle alteration in gait as the pigs walked. In the rear legs there was some medial deviation and a mild degree of over-stepping. The overstepping locomotion was accentuated by a quick downward snapping of the rear legs as they were placed on the ground. Onset of the mild paretic signs was 24 days post-treatment in 2 of the principals, which is the earliest that mild signs were seen by Kruckenberg et al (1973) in pigs percutaneously inoculated with TOCP.

The clinical findings in pigs in this study dosed with TOCP at the 500 mg/kg level differ dramatically from the results obtained by Wilson et al (In press, 1982). It should be noted that Wilson et al used older and larger pigs (average weight of 37 kg) than those employed in this study (average weight of 12.2 kg). Possibly the 500 mg/kg dosage level was just below the threshold level at which moderate or severe signs would have occurred in the smaller sized pig.

The residual background histopathologic lesions of the spinal cords of pigs in the study were comparatively more mild than those described in the hen (Bickford et al, 1981). The relative freedom of the young porcine spinal cord from background "noise" is an advantage in histopathologic evaluations in delayed neurotoxicity studies.

Hemorrhage in the gray matter of the spinal cord sections was consistently encountered in both groups. The lesions varied in severity and amount from several small foci to multiple scattered balls of hemorrhage.

Blood was present in the central canal of 1 section. Presumably, the hemorrhage was the result of using electrical shock (110 volts) for euthanasia. Since electrocution is sometimes used as a method of euthanasia in swine, its possible effects on tissues of the central nervous system should be borne in mind to avoid misinterpretation of lesions.

The lot number of TOCP used in this experiment has repeatedly produced posterior paresis in 21 days in 10 out of 10 hens used as positive controls in safety studies. TOCP used at the 500 mg/kg dosage level in pigs, however, produces only mild clinical signs of posterior paresis. But significant amounts of Wallerian degeneration in the white matter of the spinal cord of TOCP treated pigs do result.

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Table 1 - Body Weight Changes in 6 Untreated Pigs and 6 Pigs Treated with TOCP at 500 mg/kg Body Weight, Per Os

Experimental Day	Group	Mean Body Weight (kg)	Standard Deviation	Absolute Difference of Means	Significance
-10	Control	8.6	2.0	0.3	NS
-10	Treated	8.9	2.2		
0	Control	12.3	2.5	0.1	NS
0	Treated	12.2	2.6		
7	Control	13.7	3.6	0.4	NS
7	Treated	14.1	3.1		
14	Control	16.5	4.1	0.3	NS
14	Treated	16.8	3.5		
21	Control	19.9	4.9	0.4	NS
21	Treated	20.3	3.6		
28	Control	24.2	4.9	0.3	NS
28	Treated	24.5	3.9		
42	Control[1]	32.2	6.6	0.5	NS
42	Treated[2]	31.7	4.6		

NS = not significant; S = significant at the 95% confidence level

[1] In the control group the average daily gain during the 42 day period was 0.48 kg

[2] In the treated group the average daily gain during the 42 day period was 0.47 kg

Table 2 - Sequential Development of Clinical Signs Associated with Posterior Paresis In 6 Pigs Treated with TOCP at the Dose Level of 500 mg/kg of Body Weight, Per Os.

	Clinical Signs* (occurring on experimental days)				
Pig Number	Mild Signs	Moderate Signs	Posterior Incoordination	Partial Posterior Paresis	Total Posterior Paresis
T1	24	0	0	0	0
T2	25	0	0	0	0
T3	24	0	0	0	0
T4	0	0	0	0	0
T5	25	0	0	0	0
T6	0	0	0	0	0

*Sequential development of clinical signs of posterior paresis was classified into 5 groups: mild, moderate, posterior incoordination, partial posterior paresis, and total posterior paresis. Mild signs of developing posterior paresis included stationary and walking hypermetria, occasional "stringhalt" movements when walking, slight medial deviation of the toes of the rear legs when walking, and occasional knuckling-over on the fetlock joint and shifting the weight from one rear leg to the other while standing. Moderate signs included consistent and easily detected hypermetria, medial deviation of the toes of the rear legs when walking (to the point that they touched the opposite leg), frequent knuckling-over of the fetlocks (as if tripped), and falling when trying to stop or turn quickly. Posterior incoordination was a point in development of neurotoxic signs that included falling down in the rear quarters when standing. The pig could still rise by himself, but would frequently fall. Partial posterior paresis was indicated when the pig could still rise to his feet, but would fall after a few steps. If assisted with a tail lift, pigs could stand and walk a few steps. Complete posterior paresis was when the pig could not rise to his feet or stand, even if assisted to his feet by a tail lift.

Table 3 - Axonal Swelling in Spinal Cords of 6 Untreated Pigs and 6 Pigs Treated with TOCP at 500 mg/kg, Per Os

Group	Cord Level Sectioned	Number of Swollen Axons	Average Number of Swollen Axons Per Section	Standard Deviation	Significance
Control	Cervical	1	0.167	0.408	S
Treated	Cervical	12	2.000	2.280	
Control	Thoracic	3	0.500	0.837	NS
Treated	Thoracic	4	0.667	0.516	
Control	Lumbar	1	0.167	0.408	NS
Treated	Lumbar	3	0.500	0.837	
Control	Combined[1]	5	0.278	0.575	S
Treated	Combined[2]	19	1.056	1.514	

NS = not significant; S = significant at the 95% confidence level

[1] In the control group 4 out of 6 pigs were affected and 4 out of 18 cord sections were affected

[2] In the treated group 5 out of 6 pigs were affected and 10 out of 18 cord sections were affected

Table 4 - Myelin Sheath Distension in Spinal Cords of 6 Untreated Pigs and 6 Pigs Treated with TOCP at 500 mg/kg Body Weight, Per Os

Group	Cord Level Sectioned	Number of Distended Myelin Sheaths	Average Number of Distended Sheaths Per Section	Standard Deviation	Significance
Control	Cervical	1	0.167	0.408	S
Treated	Cervical	7	1.167	1.169	
Control	Thoracic	1	0.167	0.408	NS
Treated	Thoracic	2	0.333	0.516	
Control	Lumbar	1	0.167	0.408	NS
Treated	Lumbar	0	0	0	
Control	Combined[1]	3	0.167	0.383	NS
Treated	Combined[2]	9	0.500	0.857	

NS = not significant; S = significant at the 95% confidence level

[1] In the control group 2 out of 6 pigs were affected and 3 out of 18 cord sections were affected

[2] In the treated group 4 out of 6 pigs were affected and 6 out of 18 cord sections were affected

Table 5 - Additional Histopathologic Changes in Cervical, Thoracic, and Lumbar Spinal Cords of 6 Untreated Pigs and 6 Pigs Treated with TOCP at 500 mg/kg Body Weight, Per Os

Parameter	Group	Number of Pigs Affected[1]	Number of Cord Sections[2]	Percent of Cord Sections	Significance
Lymphocytic perivascular cuffing	Control	2/6	3/18	16.7	S
Lymphocytic perivascular cuffing	Treated	0/6	0/18	0	
Glial cell proliferation	Control	1/6	1/18	5.6	NS
Glial cell proliferation	Treated	2/6	2/18	11.1	
Neuronal swelling and chromatolysis	Control	0/6	0/18	0	NS
Neuronal swelling and chromatolysis	Treated	0/6	0/18	0	
Vacuolization of white matter	Control	0/6	0/18	0	NS
Vacuolization of white matter	Treated	0/6	0/18	0	
Hemorrhage in gray matter	Control	6/6	16/18	88.9	NS
Hemorrhage in gray matter	Treated	6/6	17/18	94.4	

NS = not significant; S = significant at the 95% confidence level

[1]Number of pigs with at least 1 lesion in a spinal cord cross-section/number of pigs in the group

[2]Number of individual cross-sections affected with at least 1 lesion/number of individual cross-sections examined. In each pig, 1 spinal cord cross-section was examined at 3 different anatomic locations (mid-cervical, mid-thoracic, and mid-lumbar)

APPENDIX

APPENDIX 1: Parameters Used in Evaluating and Recording Changes in Pigs Treated with Tri-o-tolyl Phosphate (TOTP)

Parameter	Time in hours - post exposure						
	.25	.5	1	2	4	8	16
POSITION							
Standing							
Sternal recumbency							
Lateral recumbency							
Lateral recumbency-paddling							
Comatose							
NEUROMUSCULAR							
Muscle trembling							
Tremors of front legs							
Tremors of rear legs							
Shaking all over							
Unsteadiness of front legs							
Unsteadiness of rear legs							
Unsteady while standing							
Unsteady while walking							
Incoordination front legs							
Incoordination rear legs							
Posterior ataxia/paresis							
Anterior ataxia/paresis							

APPENDIX 1: Continued

Parameter	Time in hours - post exposure						
	.25	.5	1	2	4	8	16
GASTRO-INTESTINAL							
Normal formed stool							
Loose stool							
Watery stool							
Propulsive diarrhea							
Eating							
Drinking							
CLINICAL APPEARANCE							
Normal							
Restless							
Depressed							
Salivating							
Shaking head							
Moving tongue							
Discharge from mouth							
Discharge from nostrils							
RESPIRATORY							
Normal							
Coughing or gagging							
Dyspnea							
Increased depth							
Hyperventilation							

APPENDIX 2 : Parameters Used to Monitor Development of Delayed Neurotoxicity in Pigs Treated with Tri-o-tolyl Phosphate (TOTP)

Parameter	Days - post exposure													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Normal alertness														
Can stand														
Cannot stand														
Normal locomotion														
Sternal recumbency														
Lateral recumbency														
Muscle tremors/convulsions														
Stationary and walking hypermetria														
Occasional "stringhalt" movements when walking														
Slight medial deviation of toes of rear legs when walking														
Occasional knuckling-over on fetlock joint														
Consistent and easily detected hypermetria														
Medial deviation of toes of rear legs														
When walking														
Frequent knuckling-over on fetlock joint														
Falling when trying to stop or turn quickly														
Falling down in rear quarters when standing														
Pig able to rise voluntarily but falls after a few steps														
"Dog-sitting" position when resting, eating, or drinking														
Pig able to stand and walk when assisted with tail-lift														
Pig unable to stand even when assisted with tail-lift														
Comatose														
Death														

APPENDIX 2 : Continued

Parameter	Days - post exposure										
	15	16	17	18	19	20	21	22	23	24	25
Normal alertness											
Can stand											
Cannot stand											
Normal locomotion											
Sternal recumbency											
Lateral recumbency											
Muscle tremors/convulsions											
Stationary and walking hypermetria											
Occasional "stringhalt" movements when walking											
Slight medial deviation of toes of rear legs when walking											
Occasional knuckling-over on fetlock joint											
Consistent and easily detected hypermetria											
Medial deviation of toes of rear legs											
When walking											
Frequent knuckling-over on fetlock joint											
Falling when trying to stop or turn quickly											
Falling down in rear quarters when standing											
Pig able to rise voluntarily but falls after a few steps											
"Dog-sitting" position when resting, eating, or drinking											
Pig able to stand and walk when assisted with tail-lift											
Pig unable to stand even when assisted with tail-lift											
Comatose											
Death											

APPENDIX 2: Continued

Parameter	Days - post exposure										
	26	27	28	29	30	31	32	33	34	35	36
Normal alertness											
Can stand											
Cannot stand											
Normal locomotion											
Sternal recumbency											
Lateral recumbency											
Muscle tremors/convulsions											
Stationary and walking hypermetria											
Occasional "stringhalt" movements when walking											
Slight medial deviation of toes of rear legs when walking											
Occasional knuckling-over on fetlock joint											
Consistent and easily detected hypermetria											
Medial deviation of toes of rear legs											
When walking											
Frequent knuckling-over on fetlock joint											
Falling when trying to stop or turn quickly											
Falling down in rear quarters when standing											
Pig able to rise voluntarily but falls after a few steps											
"Dog-sitting" position when resting, eating, or drinking											
Pig able to stand and walk when assisted with tail-lift											
Pig unable to stand even when assisted with tail-lift											
Comatose											
Death											

APPENDIX 2 : Continued

Parameter	Days - post exposure					
	37	38	39	40	41	42
Normal alertness						
Can stand						
Cannot stand						
Normal locomotion						
Sternal recumbency						
Lateral recumbency						
Muscle tremors/convulsions						
Stationary and walking hypermetria						
Occasional "stringhalt" movements when walking						
Slight medial deviation of toes of rear legs when walking						
Occasional knuckling-over on fetlock joint						
Consistent and easily detected hypermetria						
Medial deviation of toes of rear legs						
When walking						
Frequent knuckling-over on fetlock joint						
Falling when trying to stop or turn quickly						
Falling down in rear quarters when standing						
Pig able to rise voluntarily but falls after a few steps						
"Dog-sitting" position when resting, eating, or drinking						
Pig able to stand and walk when assisted with tail-lift						
Pig unable to stand even when assisted with tail-lift						
Comatose						
Death						

CLINICAL SIGNS AND HISTOPATHOLOGIC CHANGES OF THE SPINAL CORD IN PIGS TREATED WITH TRI-O-CRESYL PHOSPHATE

by

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ABSTRACT

The intent of this study was to determine if pigs, given the standard hen test dose of the organophosphate, tri-o-cresyl phosphate (TOCP), would develop clinical signs of delayed neurotoxicosis of the type and severity produced in the chicken. Also, histopathologic sections from 3 different levels of the spinal cord were to be examined for possible changes induced by the drug.

Six female Yorkshire pigs (average weight of 12.2 kg) received a single oral dose of TOCP of 500 mg/kg. Six other Yorkshire gilts of similar weight and size received no treatment. The pigs were observed for acute toxicosis at .25, .5, 1, 2, 4, 8, and 16 hours following treatment and then daily thereafter for clinical signs of delayed neurotoxicosis. Weights of both groups were recorded weekly during the course of the 42 day experimental period.

At the end of the 42 day period, all 12 pigs were euthanatized via electrocution (110 volts). Necropsies were performed on all pigs, and the spinal cords were fixed in 10% buffered neutral formalin. Three spinal cord sections from each pig (C_4, T_7, and L_4) were prepared and stained with hematoxylin and eosin.

There were no statistically significant differences between the weights of the groups at any of the weighing periods. None of the pigs developed signs of acute toxicosis.

Four of the 6 treated pigs showed mild clinical signs of posterior paresis (mild overflexion of the hock and tendency toward medial deviation of the rear limbs) between 24 and 25 days post-exposure. Through the remainder of the experimental period, signs in the 4 affected pigs did not advance beyond the mild stage.

The spinal cord cross-sections from each pig were examined by light microscope. Statistically, the treated group had a significantly greater number of swollen axons and distended myelin sheaths than the control group. These statistically significant differences existed only at the mid-cervical (C_4) level.