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Case Report - Disulfoton Poisoning of Beef Cattle

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Abstract

A herd of beef cattle routinely fed cotton industry by-products was poisoned by disulfoton-treated cotton seed that was disposed in cotton gin trash. Disulfoton is an organophosphorus insecticide. Eighteen of 48 animals died. Blood acetylcholinesterase (AChE) levels were used to determine clearance of disulfoton from the surviving animals, and to determine when the animals could be sold. At 36 days post-exposure three animals, although asymptomatic, had AChE levels that suggested persistent AChE inhibition. At 77 days following initial exposure, AChE levels were within normal limits.

Résumé

Un troupeau de bovin de boucherie nourri régulièrement avec des sous-produits de l'industrie du coton a été empoisonné par des graines de coton traitées au disulfoton qui étaient jetées dans des égreneuses de coton. Le disulfoton est un insecticide organophosphoré. Dix-huit individus sur 48 sont morts. La concentration sanguine d'acétylcholine estérase (AChE) a été utilisée pour déterminer la clairance du disulfoton chez les sujets vivants et déterminer à quel moment les individus pourraient être vendus. Trente six jours suivant l'exposition, trois individus montraient de façon asymptomatique une concentration d'AChE qui suggérait une inhibition permanente d'AChE. Soixante dix sept jours suivant l'exposition initiale, les niveaux de l'AChE étaient à l'intérieur des limites normales.

Introduction

Disulfoton is an organothiophosphorus compound in the family of organophosphorus (OP) insecticides that is primarily used for agricultural purposes. Development of OP insecticides occurred secondary to development of the toxic war gases sarin, tabun and soman during World War II.[2,3,9] These compounds are among the most toxic chemical warfare agents known.[2] Use of OP insecticides in agriculture increased dramatically following the war, with great economic benefit to all aspects of the industry. Although still in use, the amount of disulfoton used in agriculture has significantly declined since the 1970s.[11]

Disulfoton is used in agriculture for insect control.[11] One example of its use is the impregnation of seed grain with disulfoton to control insect damage during storage, planting and germination. Disulfoton and captan, a fungicide of low toxicity (rat oral LD_{50}-9000 mg/kg),[1] are the active ingredients of Di-Syston®.[a]

Disulfoton has been administered orally to calves at 0.11 mg/lb (0.25 mg/kg) and yearlings at 0.22 mg/lb (0.5 mg/kg) without toxic effects. The maximum non-toxic oral dose for sheep and goats is 0.45 mg/lb (1 mg/kg).[6]

Organophosphorus compounds competitively inhibit acetylcholinesterase (AChE) by phosphorylation of the acetylcholine (ACh) receptor site, allowing a build up of ACh at the neuron-to-neuron or neuron-to-target organ junction. Inhibition of AChE due to exposure to OPs is considered irreversible in most instances. Acetylcholine mediates the transmission of nerve impulses between pre- and postganglionic neurons in the autonomic nervous system. Acetylcholine also mediates nerve impulses from somatic nerves to skeletal muscle, and some neuron-to-neuron junctions within the central nervous system (CNS) are also mediated by ACh. Uncontrolled accumulation of ACh causes over-stimulation of the target organ and over production of the product of that organ.

Organophosphorus insecticides are readily absorbed via oral, dermal, inhalation and mucous membrane exposure.[8] They are readily distributed throughout the body, but do not accumulate in fat.[8] The effects of OP toxicity are classified as muscarinic, nicotinic or CNS. The muscarinic effects occur most often and include salivation, gastrointestinal hypermotility

[a]Bayer Corporation Agriculture Division, Kansas City, Mo. 64120-0013

with pain, diarrhea often containing flecks of blood, lacrimation, miosis, dyspnea, micturition and bradycardia.[4] Nicotinic effects, which occur less often, cause skeletal muscle tremors, tetany and paralysis. The CNS effects of depression, seizures and central respiratory paralysis rarely occur.[4,8]

Death most often results from hypoxia associated with the muscarinic effects of bronchial constriction and increased bronchial secretions, which occlude the airways, in conjunction with bradycardia. Occasionally, the nicotinic effects lead to paralysis of the respiratory muscles due to fatigue and, rarely, respiratory arrest can occur as a result of the CNS effects.[2,9]

Previous exposure to other drugs that inhibit AChE, such as phenothiazine tranquilizers, succinylcholine and quaternary ammonium compounds, potentiate the effects of OP insecticides.[2,3,8,9] Organophosphorus compounds that contain the thiophosphate ester have sulfur bound to the phosphorous molecule (P=S bond) instead of oxygen (P=O bond). These organothiophosphorus compounds are generally less toxic than those that have the P=O bond.[11] When organothiophosphorous compounds are oxidized by the mixed-function oxidase system, the sulfur is replaced with oxygen and toxicity increases.[8]

Disulfoton is oxidized by hepatocytes forming two definitive metabolites, demeton S-sulfoxide and demeton S-sulfone, which are eliminated in urine.[5,11] These metabolites can be further degraded to diethylphosphoric acid or diethyl-phosphorothiolate, which can also be found in the urine.[5]

Although not exclusive for OPs, determination of OP toxicity is based on measuring blood or brain AChE levels.[2,3,7,9] A marked decrease in blood or brain AChE activity is highly suggestive of OP or carbamate toxicosis, with the exception of exposure to the blue-green algae, *Anabaena flos-aquae*. This algae is occasionally present in livestock water sources and should be included in the differential diagnosis when environmental conditions favor its growth. It contains a neurotoxin, antitoxin a, that inhibits blood but not brain AChE.[2]

The unit of measure for AChE activity is delta pH/hr. This value represents the change in pH of the brain or blood sample before and after the addition of ACh. A water blank and a blood or brain sample from a normal animal are tested at the same time. Acetylcholinesterase that is bound by the phosphorus group of an OP compound is unable to hydrolyze the ACh and very little or no change in pH will occur, similar to the water blank. If the AChE is not inhibited, then the added ACh will be hydrolyzed to choline and acetic acid, resulting in a significant decrease in pH.[7] If the delta pH value of the test sample is less than 25% of the control, exposure to an OP or carbamate insecticide is likely.[2]

Postmortem lesions that occur with OP poisoning are not diagnostic. Pulmonary edema with increased bronchial secretions, dilation of the gastrointestinal tract and serosal and mucosal gastrointestinal hemorrhages can be present.[2,8]

Recommended treatment for toxicity due to ingestion of contaminated feed includes the administration of atropine sulfate at 0.22 mg/lb (0.5 mg/kg), with 25% of the dose given intravenously and the remaining 75% administered subcutaneously or intramuscularly every three to four hours for 1 to 2 days.[10] Activated charcoal (1.36 g/lb; 3 gm/kg) administered orally is indicated to bind any unabsorbed OPs present in the gut.[10] In valuable animals, administration of oximes such as protopam (2 PAM) is recommended. 2 PAM should be dosed at 9mg/lb (20 mg/kg) every 12 hours until clinical signs are no longer present.[2]

Some OPs undergo a process known as "aging" within the animal. Aging strengthens the AChE-phosphorus bond, rendering oxime therapy ineffective.[2] When aging occurs, replenishment of the phosphorylated AChE is by synthesis of new AChE over a 20-30 day period.[3] If no improvement is seen after four doses of oxime therapy, the value of further treatment with this drug is questionable and discontinuation should be considered.[2]

Case History and Clinical Findings

A cattleman routinely used cotton gin trash (CGT) for winter supplementation of his beef herd which consisted of 30 cows, 17 calves and one bull. Cotton gin trash is the remainder after the lint and seed have been removed from the cotton boll. The CGT was purchased from a local cotton gin and fed free-choice on the ground. Two days after purchasing a new load of CGT, 11 cows were found dead and six others were critically ill. Clinical signs of those critically ill included salivation, ataxia, watery diarrhea, muscle tremors, severe depression, weakness and inability to rise. Most of the remaining herd exhibited diarrhea.

Examination of the recently purchased CGT revealed cottonseeds that had been dyed purple (Figure 1). This finding, plus the observed clinical signs, supported a tentative diagnosis of OP poisoning. Six more cows and a steer calf subsequently died. Postmortem examination of the dead cows did not reveal significant lesions. Liver, kidney, and stomach content, along with a blood sample preserved from an affected sick cow, were submitted to the Oklahoma Animal Disease Diagnostic Laboratory (OADDL) for toxicological analysis.

Analysis of the blood sample at the OADDL revealed significantly depressed blood AChE activity, with a delta pH/hr measurement of 0.02/hr. The water blank measured 0.03 delta pH/hr and the blood control measured 0.77 delta pH/hr. These test results supported the tentative diagnosis of OP poisoning. Analysis of rumen content by gas chromatograph/mass spectrometry (GC/

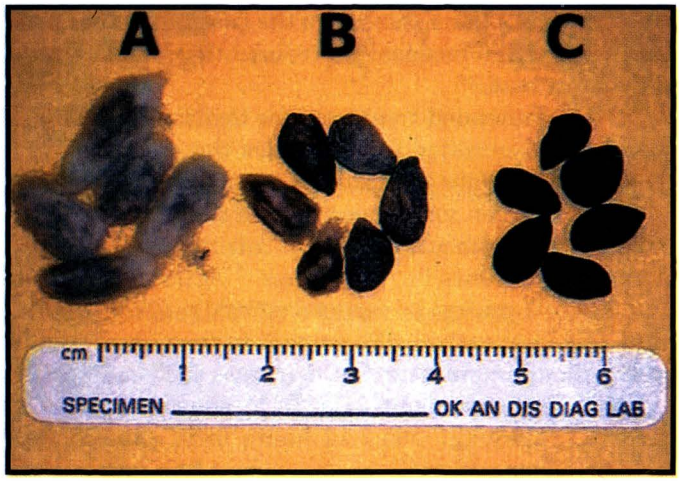

Figure 1.
A - Cottonseed with lint
B - Cottonseed without lint
C - Cottonseed dyed purple

MS) detected disulfoton at 32 mg/lb (70.5 mg/kg). The purple colored cottonseed recovered from the pile of CGT at the farm contained 16.7 mg/lb (36.72 mg/kg) of disulfoton. The rumen content and cottonseed were analyzed independently using different methods; GC/MS Quadrupole was used to analyze the rumen content and GC/MS Ion-Trap was used to analyze the cottonseed.

When the owner of the cotton gin was contacted, it was determined that 26 bags of cottonseed treated with Di-Syston® had been disposed in the CGT.

Case Discussion

Eighteen of the 48 animals died. The insurance carrier for the cotton gin settled with the rancher by paying for the dead animals and purchasing the 30 remaining animals. The insurance company then contacted the OADDL toxicology department for recommendations for disposition of these animals. Although most OP compounds are rapidly eliminated from the body with minimal tissue or milk residue within hours of exposure, it was recommended that blood AChE levels be determined on the surviving 30 animals to evaluate the exposure of each individual animal. Blood samples were taken 36 days after initial exposure to the disulfoton (Table 1). At that time only three mature cows had blood delta pH/hr values suggestive of persistent AChE inhibition. It was recommended that those three cows be re-evaluated in 30 days. When re-evaluated 41 days later, all three cows had delta pH/hr values greater than 45% of the control. During the interval between sampling periods no adverse signs were noted, and two cows delivered normal, healthy calves. When the last three cows had normal delta/pH values, the insurance company sold the cattle.

Table 1. Blood cholinesterase activity of surviving cows 36 days after exposure to disulfoton

Animal ID	Delta pH/hr	Animal ID	Delta pH/hr	Animal Id	Delta pH/hr
76	0.53	86	0.66	96	0.73
77	1.02	87	0.62	97	0.91
78	0.83	88	0.98	98	0.87
79	0.85	89	0.23[a]	100	0.80
80	0.26[a]	90	0.64	101	0.57
81	0.34[a]	91	0.67	102	0.82
82	0.62	92	0.98	103	1.14
83	1.16	93	0.68	104	0.53
84	0.45	94	0.93	105	0.71
85	0.76	95	0.82	106	0.76

[a]Denotes values between 25 - 30% of control value

Table 2. Blood cholinesterase of 3 cows 77 days after exposure to disulfoton

Animal ID	Delta pH/hr
80	0.35
81	0.50
89	0.41

Conclusions

Ruminant livestock can effectively utilize many by-products from other food and agricultural industries. In many instances by-products are attractively priced, because to some extent they are otherwise "waste." As illustrated in this case report, it is imperative that each shipment be carefully examined for the presence of foreign material, unusual odors or dyed materials. Any abnormal findings should be investigated before by-products are fed to livestock.

Large numbers of sick cattle or deaths in a short period of time is highly suggestive of feed or water contamination. Prompt movement of cattle to different feed and water sources is essential to minimize continued losses. When poisoning is suspected, a toxicologist should be contacted immediately to determine what samples should be taken for analysis, and to ascertain how samples should be preserved.

All necessary steps should be taken to avoid marketing any animals which may have violative residues or in any way pose a food safety threat.

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Patterns of parasitic nematode infection and immunity in dairy heifers treated with ivermectin in a sustained-release bolus formulation either at turnout or in the middle of the grazing season

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Twenty-eight Holstein-Friesian heifers, born the previous year and weighing between 130 and 310 kg, were allocated to one of two treatment groups by restricted randomisation, based on their initial weight The heifers in group 1 were each treated with ivermectin in a sustained-release bolus formulation at turnout in April, and those in group 2 were each given an ivermectin bolus on July 10, 84 days after turnout. On that day the mean geometric worm egg counts of groups 1 and 2 were 0·4/g and 38·8/g respectively, and they both had a mean plasma pepsinogen concentration of 0·59 iu/litre; in group 1, two of 14 faecal samples were positive for *Dictyocaulus viviparus* larvae, and in group 2 all 13 samples were positive; in group 1 eight calves were positive and three inconclusive for the presence of antibodies to *D viviparus,* and in group 2 the corresponding figures were 10 positive and two inconclusive; the mean liveweights of groups 1 and 2 were 274·4 kg and 262·8 kg, respectively. By December 4, 231 days after turnout, the corresponding results were: mean geometric worm egg counts of 2·2/g and 0·5/g; one of 13 and none of 14 faecal samples positive for *D viviparus* larvae; 12 positive and two inconclusive and none positive and 10 inconclusive for the presence of antibodies to *D viviparus;* 214 days after turnout their mean liveweights were 361·1 kg and 358·3 kg. Although the patterns of parasitic nematode infection were different in the two groups during the grazing season, by the time they were housed both groups had achieved similar liveweights and showed evidence of an immune response to both *D viviparus* and gastrointestinal nematodes.