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The Diagnostic Approach and Public Health Implications of Phorate Poisoning In a California Dairy Herd

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Abstract

Within an hour of the planned feeding of a group of 300 Holstein cattle, a large number of cattle developed tremors, diarrhea, weakness, and paralysis. All cows exposed to the feed, a total of 159, died within 24 hours despite treatment attempts with atropine in approximately 100 animals. An additional 8 exposed animals were culled within a week. A thorough investigation demonstrated the accidental mixing of phorate into the total mixed ration instead of the intended mineral mix. Diagnostics confirming phorate exposure in deceased animals included brain cholinesterase determinations and the analysis of liver, rumen content, milk, and feed for phorate. Phorate is a restricted-use organophosphorus pesticide commonly used in US agriculture because of target pest efficacy, cost, and availability. Even with known human, animal, and ecological risks, organophosphorus pesticide exposure in food animals and the corresponding public health risks for humans, including the potential for meat and milk residues and exposure in children. This report describes a dairy's catastrophic loss of 167 cows caused by human error and assesses the public health implications therein. Veterinary diagnosticians, public health officials, and veterinarians must be prepared to collaborate in order to advise clients on case work-up, management, and preventive measures.

Keywords: Bovine; Dairy; Diagnosis; Organophosphorus; Pesticide; Phorate; Poisoning; Public health; Residue; Toxicosis

Introduction

Organophosphorus Pesticides (OPs) are a group of synthetic phosphorus compounds that were developed into insecticides in the 1940s and 1950s. They remain the most widely used insecticides in the world because of their ability to control a wide variety ofpests and because most OPs do not persist in the environment. The major mechanism of toxicity of OPs is the inhibition of Acetylcholinesterase (AChE), resulting in a net accumulation of Acetylcholine (ACh) and increased stimulation of cholinergic receptors [1]. In mammals, excessive stimulation of these cholinergic receptors in the central and peripheral nervous systems results in a well-described series of clinical signs manifested by muscarinic-receptor induced effects (excessive secretions, miosis, bradycardia) and nicotinic-receptor-induced effects (muscle tremors, convulsions, complete muscle paralysis). The course of OP poisoning is, as is true for all toxicants, influenced by the amount ingested and the duration of exposure. Typically an acute intoxication is observed with onset of clinical signs within minutes of exposure. Organophosphorus pesticide poisoning in cattle occurs frequently enough that itshould be considered as a differential diagnosis in animals presented with signs consistent with cholinergic toxicity [2-4].

Case Description

The current report describes a case of OP poisoning in dairy cows. Information regarding affected cows' history, clinical signs, and diagnostic work-up is detailed. In addition, risks for milk and meat residues, public health risks, and measures for prevention and management of OP exposure are discussed. Three hundred Holstein animals of a 600 cow dairy located in Kings County in the Central Valley of California were fed a total mixed ration (TMR; individual commodities not available) one morning after milking (Day 1). The ration was intended for lactating and dry cows and heifers, and cows in the hospital pen. After having fed 167cows, the mixer wagon had a flat tire and feeding of the remaining cows was postponed. When the feeder stepped off the truck, he noted several dead cows in the pen he had fed minutes prior. In addition, other cows that had been fed were showing signs of tremors, foaming at the mouth, weakness, inability to stand, and collapse. The veterinarian was contacted immediately and arrived shortly after the onset of clinical signs, at which time 10 animals had already died. Because the clinical signs were suggestive of cholinergic toxicity, atropine was administered intramuscularly to approximately 100 clinically affected cows. Despite treatment, 70/167 cows had died by 12 PM on Day 1. By the evening of Day 1, 150/167animals were dead. A total of 159 cows that had been offered the TMR on the morning of Day 1 were dead within 24 hours (Figure 1). Eight exposed cows that survived the initial poisoning developed decreased milk production in the following days and were culled on Day 8. The cows on the dairy that were not fed from the mixer truck on the morning of Day 1 remained clinically normal.

Post-mortem evaluation of three cows in the field on Day 1 revealed no specific lesions, but a garlic-like odor was detected in the rumen contents. Liver, rumen contents, and heads were submitted from all

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Figure 1: Cows that died acutely from phorate toxicosis in the Central Valley of California, USA: 159/167 cows died within 24 hours of exposure with clinical signs of cholinergic toxicity

3 cows to the California Animal Health and Food Safety Laboratory for testing. One milk sample and three feed samples (two total mixed ration samples and one sample of almond hulls) were also submitted for toxicological evaluation. Upon delivery of the feed samples to the toxicology laboratory, a strong sulfur-like odor was detected. Because of the rapid onset of clinical signs, high mortality, and the strong odor of feed samples, exposure to a sulfur-containing organophosphorus insecticide was considered as a top differential. Within hours of sample submission to the laboratory, three brain samples, sagittal sections of 1/2 the brain, were examined for cholinesterase activity using a modification of the Ellman method [5]. The brain acetylcholinesterase activities were 0.32, 0.35, and 0.41 μ M/g/min, consistent with severe depression when compared to adequate mean brain AChE activity of $3.78 \ \mu M/g/min$ for cattle [6]. Because of the reduction in brain AChE activity, three feed and three rumen content samples were immediately analyzed for 11 carbamate and 44 organophosphorus insecticides by gas chromatography-flame ionization detector with gas chromatographymass spectrometry confirmation [7]. All samples contained phorate. Phorate concentrations in the total mixed ration samples were 50 and 1800 mg/kg (on a wet weight basis), and 1900 mg/kg (wet weight) in the almond hulls. The rumen contents contained 28.5, 13.5 and 29 mg/kg (wet weight) of phorate. A bulk milk tank sample collected on Day 1 was negative for phorate at a limit of detection of 10 ng/ml (ppb). Muscle tissue and livers collected from 3 cows that were culled on Day 8 after exposure were negative for phorate at the method detection limits of 50 ng/g (ppb; wet weight) and 100 ng/g (ppb wet weight), respectively.

Phorate (Thimet 20G ^{••}) was identified as the cause of this massive mortality event. Phorate was found in the almond hulls that were incorporated into the total mixed ration that morning. Even after weeks of intensive investigative efforts, the following remained undetermined: why phorate-contaminated almond hulls were fed to the cows; whether these almond hulls were originally intended to be fed to the cows; and whether there was malicious intent that led to phorate toxicosis. It was concluded that a bag of phorate that was stored on top of the almond hulls was likely mistaken for a bag of mineral mix. When almond hulls were scooped up by the feeder, the adjacent bag of phorate was punctured and its contents mixed with the hulls.

Discussion

Toxicological testing confirmed phorate toxicosis within 24 hours

of the onset of this high-mortality event on the dairy. Phorate, O,Odiethyl S-[(ethylthio) methyl]dithiophosphate, is an organophosphorus insecticide used for the control of a large variety of agricultural insect pests on beans, corn, cotton, hops, potatoes, radishes, lilies, sorghum, and sugar beets [8]. In the USA, phorate is available as Thimet [™] and is classified as a restricted use pesticide because of its high toxicity to birds, fish, and reptiles. The reported oral lethal dose 50 (LD50) in rats is 1.6 mg/kg bodyweight, placing the compound in the highly toxic category [9]. In addition, phorate is highly toxic via dermal absorption with a reported dermal LD50 in rabbits of 2.5 mg/kg bodyweight. Because of phorate's significant potential for volatilization, inhalation also represents a significant route for toxic exposure in humans and animals [10]. After absorption, phorate is rapidly distributed throughout the body and, due to its lipophilicity (log P = 3.71) [11], partitions into adipose tissues such as brain, liver, and fat. After ingestion, dermal absorption, or inhalation, phorate is oxidized to phorate oxon, phorate oxonsulfoxide, and phorate oxonsulfone via cytochrome P-450-dependent mono-oxygenases and FAD-containing mono-oxygenases [12]. These oxon metabolites of phorate are more toxic than the parent compound, resulting in significant increase in ACh inhibition. Bioactivation takes place primarily in the liver but also in extrahepatic tissues [13]. Ester cleavage is a minor pathway for phorate biotransformation. While unique biotransformation pathways have not been conducted in cattle, it is likely that metabolites identified in other mammalian species would also be formed in the bovine, such as the three highly toxic oxon derivates. However, rumen metabolism of phorate may result in detoxification or in the production of other, biologically active metabolites. The role of rumen mircorganisms in the role of biotransformation of phorate has not been examined.

There are very few studies that focus on the detection of phorate and its metabolites in edible tissue of animal origin even though there is risk to humans for low level exposure to phorate through the food supply [14-18]. In the reported mortality event on the dairy, a bulk milk tank sample was collected on Day 1 and found to be negative for phorate at a limit of detection of 10 ng/ml. While multiple bulk tank milk samples were collected for several days after the incident, no additional testing was performed after the initial milk sample from Day 1 was negative for phorate. The limit of detection of 10 ng/ml is similar to but below a recently reported detection limit of 14 ng/ml for phorate in milk [18]. The lack of detection of phorate in the bulk tank milk sample from Day 1 may be because mixing of phorate-contaminated with negative milk samples diluted the final phorateconcentration below the method detection limit. In addition, it is possible that there was insufficient time for phorate to transfer into milk prior to the death of cows or that phorate was rapidly metabolized to other unmeasured compounds. There are limited data on the degradation of organophosphorus pesticides during processing such as heat treatment [19-22]. Specific data on phorate degradation under routinely used pasteurization methods in the US does not exist. Phorate has been detected in homogenized and pasteurized Mexican milk samples at concentrations from 48 to 172 ng/ ml, with some samples exceeding the Mexican maximum residue limit of 50 ng/ml [18].

While phorate's primary mechanism of toxicity is inhibition of cholinesterase activity, other toxic effects, such as DNA damage and cellular toxicity have been described [23]. These mechanisms, considered critical in the evaluation of phorate's potential health risk after low dose, chronic exposure, can lead to neurotoxicity, carcinogenicity, and reproductive toxicity. While the high mortality case described here illustrates the typical acute intoxication from cholinergic toxicity, exposure of cattle to organophosphorus pesticides

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through veterinary drugs such as dichlorvos (Prozap TM), trichlorfon (Panacur TM) and crufomate (Ruelene TM) for the control of external parasites such as flies and grubs or through accidental exposure (e.g. off-label use; aerial spraying; feed contamination) cannot be ignored. Although over 1,398 domestic food commodities were analyzed for 473 pesticide residues in 2008 [24] by the Center for Food Safety and Applied Nutrition of the US Food and Drug Administration, only 6 dairy products were included in the annual testing, 4 cheese and 2 fluid milk samples. While none of the 6 dairy products had violative residue levels, 2 of the 4 cheese samples had detectable residues. However, the report does not identify the specific pesticides detected in these cheeses. The low number of dairy products evaluated for pesticide residues is surprising, considering that, in 2007 in the USA, nine million cows produced an average of 19,000 pounds of milk per day for a total of 171 billion pounds of milk annually. Based on data of the US Department of Agriculture, 1/3 of the produced milk is marketed as fluid milk while the remaining 2/3 are used for butter and cheese products [25].

Currently, organophosphorus insecticides make up 35% of all insecticides used in the USA [26]. Pesticide use decreased overall in the USA and OPs accounted for a diminishing share of total insecticide use (67% and 40% in 1994 and 2004, respectively) between National Health and Nutrition Examination Survey (NHANES II) and NHANES 2003-2004 [26]. However, recent data suggests that reduction in residential use of OPs was primarily responsible for decreased exposure, as little change in agricultural use of OP pesticides has occurred [27]. According to data from the California Department of Pesticide regulation, pesticide use increased in 2010 by 9.5% or 15 million pounds compared to 2009 [28]. Twelve million pounds of the reported increase was from agricultural application. In addition, the amount of OPs as well as the number of acres to which OPs were applied increased in 2010 [28]. Thus, the continued widespread and potentially increasing use of OPs in agriculture continues to pose an exposure risk to food animals and to the humans that consume animal derived products. Organophosphorous insecticides must receive special attention because of their high lipophilicity and ability to be excreted into milk.

Even when tissues are tested for OPs, results must be interpreted with caution. A study in dairy goats has shown that less than 0.02% of phorate to which an animal is exposed is actually identified as phorate in milk, liver, muscle and fat samples [29]. More than 95 to 98% of phorate in these tissues is metabolized, primarily to a methylated dephosphorylated metabolite, ethylsulfonylmethylsulfonyl methane. The applied gas chromatographic analysis designed to detect phorate as the parent compound is not suitable for the detection of phorate metabolites. Lack of knowledge of which phorate metabolites are present in cattle and the absence of analytical reference standards of phorate metabolites limit the assessment of residues. If exposure to an OP is suspected, testing of edible tissue is advisable to protect the food supply and to make informed decisions based on diagnostic test results, but negative results must be treated cautiously because of the limitations of metabolite analysis.

While diagnostic testing and residue concerns are of immediate importance in high mortality events in dairy cows, carcass disposal presents another complex issue that requires serious and careful consideration. In the presented case, 167 cows that died from ingestion of a highly toxic substance had to be disposed of safely. In order to assess proper disposal techniques, the stability and degradation properties of phorate had to be reviewed and applied to the environmental conditions at hand. Carcass disposal for such a large number of cows posed public health concerns. Initially, burying of carcasses in the ground was considered a viable option. However, the persistence of phorate and its



Figure 2: Disposal of phorate-poisoned cows by open air incineration assuring thermal degradation of phorate.

oxidized metabolites in soil is very complex [30]. High environmental temperatures and soil moisture content increase phorate degradation. Phorate is also hydrolyzed and degraded faster in alkaline conditions and soils with higher salinity as compared to neutral or acidic soils. Microorganisms in soil also play an important role in accelerating phorate degradation. Based on all these parameters, reported half-lives of phorate and its metabolites in soil range from 2-173 days, classifying phorate as moderately persistent in soil [31,32].

Persistence of phorate and its oxidative metabolites in water varies. Phorate itself has hydrolysis half-lives of 2 to 3 days and is not expected to persist or accumulate in aquatic systems. In contrast, phorate sulfoxide and phorate sulfone have half-lives in water of up to 180 days under acidic (pH 5) conditions and are more mobile. Overall, there is very limited data available on levels of phorate and its degradates in ground or surface water over time. Because of these uncertainties related to persistence in soil and/or water and a water table in the area of less than 17 feet (5 meters), burial was no longer considered appropriate. Instead, carcasses were burned (Figure 2). After consultation with a number of regulatory agencies, including the California Department of Water Resources, the California Air Resources Board, the California Environmental Protection Agency, and the local Fire Department, open air incineration was selected for disposal on site. This form of carcass disposal is a method that has been used successfully for many years [33] but must be conducted under explicit conditions. When following such procedure, an internal temperature of greater than 600°C is to be achieved so as to ensure thermal degradation of phorate. However, at temperatures of 700 to1000°C, phorate intermediates may be produced, and data on their toxicity is unknown [34]. Once temperatures exceed 1000°C, all phorate intermediates are destroyed. Temperatures such as this can be generated using advanced methods of incineration such as air curtain incineration and fixed facility incineration.

This case stresses the importance of knowing how to accurately and quickly diagnose a case of OP poisoning in cattle and how to address the public health issues that can arise from tissue residues and carcass disposal. As with any intoxication in food animals, OP exposure identification and resolution require prompt involvement of toxicology laboratories and regulatory agencies. Continued research is needed to improve the accuracy of detection of OP insecticide residues in milk and dairy products. This case provides an example on which to build

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case-based material and emergency response manuals in the veterinary and public health community.

Instrumentation used for analysis: as described in Holstege et al, [7] (OP screen, quantitation), Saturn 2000 mass spectrometer with gas chromatograph, model 3400, Varian Corp., Walnut Creek, CA was used for confirmation of phorate in all samples.

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The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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