

# An Outbreak of Thiram Poisoning on Spanish Poultry Farms

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**ABSTRACT.** Thiram-contaminated poultry feed caused soft egg shells, depressed growth and leg abnormalities in about 1 million birds. Corn previously treated with thiram and colored red was the source of the contamination as detected by gas chromatography and mass spectroscopy examinations. Standardized color-recognition for treated grain products could have avoided this situation and speaks to the international adoption of such standards.

Thiram (tetramethylthiuram disulfide,  $C_6H_{12}N_2S_4$ ) is the active ingredient of several widely used fungicide products. It is commonly utilized for treating corn and other seeds to control damping-off and emergence fungal diseases. Although thiram is not very toxic (1), treated grain occasionally finds its way into the poultry feed market and then its deleterious effects become evident even at low doses (2).

## CASE REPORT

In October and November of 1994, some chicken farmers from Catalonia (northeast Spain) began to complain to their regular poultry feed supplier because the number of eggs produced with soft shells was greatly increased, making them inappropriate for commercialization. For example, on 1 farm with a typical daily production of some 5,000 eggs, productivity was not affected but the number of soft-shelled eggs rose to approximately 4,700. Moreover, broilers had growth depression and leg abnormalities, which resulted in the more affected animals being unable to walk or stand.

When the problems first were noted, and after infectious diseases were ruled out, the feed company implicated rapidly removed the suspected batches of poultry feed and provided the farmers with new feed. Economic losses were significant, as some 30,000 laying hens and 70,000 broilers had consumed the problematic poultry feed of this company. It was later known that other Spanish feeder companies reported similar problems, so the total number of poultry affected was estimated at approximately 1 million birds.

The feed company suspected that batches of corn from one particular supplier were the origin of the toxic effects. The corn was red-colored, but the corn supplier explained to the company that the reason was the same as for red-colored wheat: the corn was from European Union stocks that had been withdrawn from human consumption because of economic reasons.

## Preliminary Chemical Analyses

Samples of suspect and control corn and

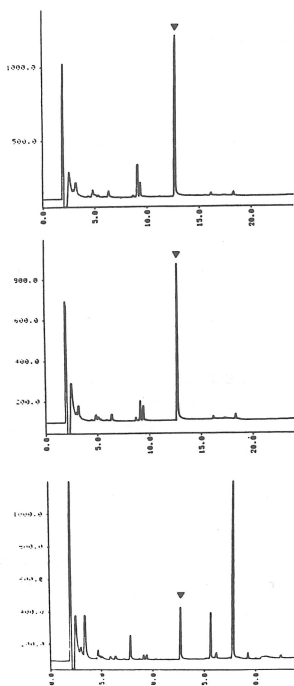
poultry feed were sent to the School of Veterinary Medicine. All samples were analyzed for organochlorine compounds by gas chromatography-electron capture detection (GC-ECD) and examined by thin-layer chromatography (TLC) under UV light and afterwards by sulfuric acid-spraying, but nothing unusual was observed except for the presence of a TLC band corresponding to the red color, which was probably Rhodamine B.

Due to the necessity for rapid results, our efforts focused on the corn samples. An old open tubular 5% phenyl-95% methylsilicone column was connected in a GC equipped with ECD, flame ionization (FID) and nitrogen-phosphorous (NPD) detectors. Corn samples were extracted successively by sonication with hexane, ethyl acetate and methanol, and after filtration these extracts were analyzed by GC/ECD, GC/FID and GC/NPD. Comparing the chromatographic patterns obtained, some peaks appeared, especially on ECD and NPD and were considered promising.

Ethyl acetate corn extracts were fractionated by TLC on silica plates using hexane/diethyl ether/methanol/acetic acid as eluent, and after scraping off selected zones from the silica were analyzed by GC. Fractions containing the suspected ECD and NPD peaks were localized, and more corn extracts were then processed in order to obtain concentrated samples for mass spectrometry (MS) examination.

## Thiram Analysis

These extracts were sent to the laboratory at the Hospital Clinic, where GC/MS electron impact ionization analyses on a methylsilicone open tubular column were carried out. In one of the fractions with a peak initially detected by NPD, thiram was tentatively identified by its 3 characteristic fragments:  $m/z$  88, 208 and 73. As the chromatographic standard of this product was not immediately available, a fungicide containing thiram (Ditiver TGD, KenoGard, Barcelona, Spain; 80% thiram w/w) was purchased, purified by TLC and analyzed by GC/MS, confirming the similarities in retention times and fragmentation patterns. When a pure thiram standard (Chem Service, West Chester, PA) became available, the identity of the product purified from the



**Figure 1.** Chromatographic profiles of TLC extracts of pure thiram, thiram-based commercial fungicide, and suspected corn (top, middle and bottom, respectively). Chromatographic conditions: 0.53 mm ID and 1.0  $\mu$ m film thickness 5% phenyl-95% methylsilicone column, eluted from 150 to 310 C at 6 C/min; He (carrier gas) at 3 ml/min; injector (splitless mode) at 310 C and detector (NPD) at 330 C. Retention time of thiram (arrow) = 12.5 min.

suspect corn and poultry feed samples was fully confirmed.

Thiram gave a good peak by GC/NPD from the 5% phenyl-95% methylsilicone column (Fig 1), but thiram quantification in the samples was finally carried out on a ODS2 high performance liquid chromatographic (HPLC) column using a programmed water-methanol eluent and UV-monitoring at 260 nm. The suspect corn had thiram concentrations of 1.9, 2.2, 3.0, 53, 66 and 418  $\mu$ g/g, and the poultry feed contained 1.5, 11, 26 and 32  $\mu$ g/g.

## DISCUSSION

Egg-shell thinning has been reported produced by several nutritional and environmental factors (3). In the present case, the available data pointed to a pesticide present in the red-colored corn as the cause of the outbreak. Some organochlorine compounds are well known as egg-shell thinning agents since early reports appeared in 1967 in wild bird species; however, for other pesticides the literature is older and more scarce. Thiram reports on egg-shell effects dated mainly from the 50's (4-6), while more recent studies

of this fungicide in poultry have been centered on the tibial dyschondroplasia caused by thiram (7). In our case this resulted in a delay of approximately 4-5 d before thiram was considered a serious candidate for the egg-shell abnormalities observed. This was about the same time the GC/MS, GC/NPD, TLC and HPLC-UV chemical evidence was obtained for the suspected samples.

Thiram-induced egg abnormalities in laying hens (1) can be produced from doses as low as 10  $\mu$ g/g in the diet (4,5). Higher levels (20-50  $\mu$ g/g) tend to produce more marked effects (decreased egg production, increased number of softer shelled eggs, lowered hatchability and chick abnormalities), while doses of 80-240  $\mu$ g/g result in complete cessation of hatching-egg production, a rapid increase in the number of soft-shell eggs and a high tibial dyschondroplasia incidence (4,6-8). These results agree with observations made in the present outbreak with thiram being fed at concentrations up to 32  $\mu$ g/g or with the first field case reported in Minnesota (4) involving approximately 75,000 hens.

Thiram-contaminated feed was accidentally sold and distributed to Spanish poultry farms, affecting approximately 1,000,000 chickens for a few weeks and producing dramatic economic losses. Confusion about the significance of red-colored corn was the initial cause of the problem. It is recommended that legislation be unified and that red-colored grains (corn, wheat) be recognized to indicate pesticide-treated seeds unfit for human or animal consumption. Other colors could be used to indicate situations in which grains are still appropriate for animal feed.

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## REFERENCES

1. Dalvi RR: Toxicology of thiram (tetramethylthiuram disulfide): A review. *Vet Hum Toxicol* 30: 480-482, 1988.
2. Waibel PE, Pomeroy BS, Johnson EL: Effect of arasan-treated corn on laying hens. *Science* 121: 401-402, 1955.
3. Burley RW, Vadehra DV: *The Avian Egg: Chemistry and Biology*. John Wiley & Sons, New York: 25-64, 1989.
4. Waibel PE, Johnson EL, Pomeroy BS et al: Toxicity of tetramethylthiuram disulfide for chicks, poults and goslings. *Poultry Sci* 36: 697-703, 1957.
5. Heuser GF: Feeding chemically treated seed grains to hens. *Poultry Sci* 34: 160-163, 1956.
6. Swanson MH, Waibel PE, Helbacka NV et al: Egg shell quality as affected by arasan in the diet. *Poultry Sci* 35: 92-95, 1956.
7. Vargas MI, Lamas JM, Alvarenga V: Tibial dyschondroplasia in growing chickens experimentally intoxicated with tetramethylthiuram disulfide. *Poultry Sci* 62: 1195-1200, 1983.
8. Page RK: Teratogenic activity of arasan fed to broiler breeder hens. *Avian Dis* 19: 463-472, 1975.

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Correct use of power requires knowing when to make persuasive comments...when to try to overpower others...and when to walk away--quietly. Realize that people's ideas or actions may be flawed--but people themselves aren't.