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Monocrotophos poisoning in wild mallards (*Anas platyrhynchos*)

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Abstracts

The toxicity of organophosphate arises from disruption of the nervous system due to the inhibition of cholinesterase enzymes, leading to death. Six dead mallards were found at Ansung where is one of the most popular wintering sites for migratory birds in Korea, and requested for diagnosis to Gyeonggi Veterinary Service on January of 2007. Some examinations including polymerase chain reaction (PCR) could not find any evidence of specific disease condition. However, the contents of gastrointestinal tracts of the birds contained residues of monocrotophos ranged from 31.3ppm to 294.3ppm by gas chromatography and mass spectrometry. It can be supposed that monocroptophos was responsible for the death of mallards by this results,

Key words: Monocrotophos, Poison, Wild mallard.

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Introduction

The agricultural chemicals used for the control of malady, vermin and weed are necessary in most fields of agriculture. The production and the use of those are increasing every year for expanding of agricultural products, resulting in environmental pollution.

The absorbed organophosphoric chemicals are dispersed in the every portions of the body within 30 minutes, causing acute toxicosis. These combined with the cholinesterase and restrainedits operation irreversibly, which leaded to accumulation of neurotransmitter acetylcholine in the synapsis among preganglionic fiber of sympathetic nerve and postganglionic fiber of parasympathetic and motor nerve. Finally intoxicated animals may die by muscarinic parayympathetic action and nicotinic sympathetic action of muscular nerve [1-3].

The intoxicated wild birds can show trembles, convulsions, paralysis, coma and severe nervous signs[4].

There were few reports on the toxicosis with organophosphoric chemicals to the animal in Korea. For example, there was a report related to dead white-napped cranes (*Grus vipio*) with parathion toxicosis, not monocrotophos in Chulwon region [4].

This study was conduct to investigate the death cause of the mallards wintering at Ansung, and describe the toxicity with monocrotophos.

Materials and Methods

Animals

This study examined six dead mallards wintering at the Ansung stream, Gyeonggi province in on January, 2007.

Gross and histopathological examination

We examined the outside of carcasses, and necropsy was done from respiratory tract to solid internal organs. Especially the remains of inflammatory exudate in respiratory organs such as trachea, lung, air sack and morphological change of the organs were investigated closely. Paraffin method was used after fixing with 10% formalin, and stained with H&E for microscope.

Organophosphoric agricultural chemicals examinations

The used reagents were hexane, acetonitrile, acetone (Burdick & Jackson, USA) and solid phase extraction (SPE) with florisil (1,000mg/6mℓ) (Applied Separations, USA), standard monocrotophos (Dr. Ehrenstofer, Germany) for control.

The instrument used for residual chemicals analysis was gas chromatography nitrogen-phosphorous detector (Hewlett Packard 5890, USA), Chemstation (Hewlett Packard, USA) was used for data system and the size of capillary column was HP-5(30m × 0.25mm, 0.25μm).

The condition of analysis was as follows: 1) injection temperature was 250 ℃, 2) detection temperature was 270℃, 3) oven was maintained initially for 3 minutes at 120℃, and for 5 minutes at 260℃ after increasing from at 120℃ to at 260℃, 4) carrier gas (N_2) flow rate was 1mℓ/min (split 50:1).

Also, to examine the quality of the chemicals, gas chromatography mass selective detector (Hewlett Packard 5973. USA) was used as like follows: 1) inlet temperature was 260℃ (split 50:1), 2) oven was maintained initially for 2 min at 100℃, and for 10 min at 230℃ after increasing from at 100℃ to at 230℃, 3) temperature of MS source and MS quadrupole were 230℃ and 150℃, respectively, 4) the size of capillary column was HP-35 (30m × 0.25mm, 0.25μm). 5) range of MS scan was 50-500 Amu, 6) flow rate of carrier gas (He) was 1mℓ/ min.

To make specimen for analysis of the chemicals, 5mℓ acetonitrile was added into 1g of chemical, which was sonicated for 10 min. Acetonitrile-layer was selected after adding of 3g NaCl into the specimen to make incrassate specimen, and it was solved with 1mℓ acetone. One milliliter of incrassate solution was added into SPE-FLO conditioned with 3mℓ hexane, which

was evaporated after elution with 5㎖ acetone. Final sample was injected into GC/NPD AND GC/ MSD after solving with 1㎖ acetone.

Microbiological examination

The samples from organs like liver were inoculated with blood agar and MacConkey agar contained tryptic soy broth, and incubated at 37℃ for 19-24 hours to isolate the pathogenic bacteria.

For the virus isolation, samples from brain and cecum sample were injected into the embryonated specific pathogen free eggs, and incubated for 5 days and haemagglutination test was carried out with allantoic fluid.

Reverse transcription polymerase chain reaction (RT-PCR)

RNA was extracted from allantoic fluid using QIAamp Viral RNA Mini Kit (Qiagen, Germany). The resultant RNA was dissolved in 40㎕ of RNase-free water.

Five microliter of RNA was mixed with 20㎕ of RT-PCR premixture (Bioneer, Korea) with NP gene specific primers (forward primer: 5'-TACAGATGTGCACTGAACTCAA-3' and reverse primer: 5'-CAGCAGTTGCGTCTTCTCCATT-3').

The RT-PCR was performed in Dyad (MJ reaserch, USA) and program was 50℃ for 30 min, and 94℃ for 4 min followed by 35 cycles of 94℃ for 30 sec, 50℃ for 35 sec, and 72℃ for 25 sec and lastly followed by 72℃ for 7 min.

The size of this PCR product was 273 bp and was observed over an UV transilluminator after electrophoresis in 1.5% agarose gels.

Results

Gross and histopathological findings

Health condition of dead mallards was considered as good because the subcutaneous lipid was thick, and there in no external injury (Fig 1). Lot of undigested paddies were observed in upper digestive tract such as craw, gizzard and grandular stomach (Fig 1). No pathologic and histopathological lesions were observed in the solid organs as like liver, heart, lung, kidney and spleen.

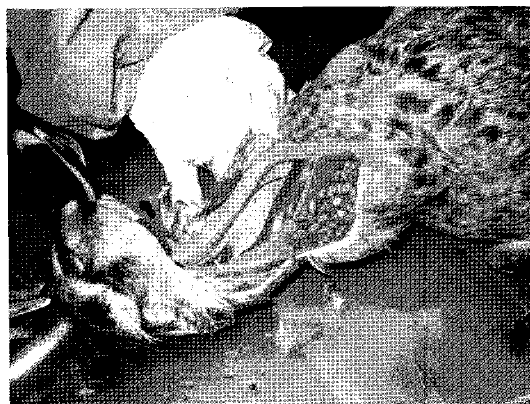

Fig 1. Lot of paddies in the crop of a mallard

The result of organophosphoric chemicals examination test

The samples from gastrointestinal tract, liver and kidney were analyzed with GC/NPD, respectively for quantity. The result indicated that the retention time was consistent with that of Monocrotophos (Fig 2). To confirm the spectrum of monocrtonphos, we examined the chemicals with the GC/MSD, and the chromatogram was same as

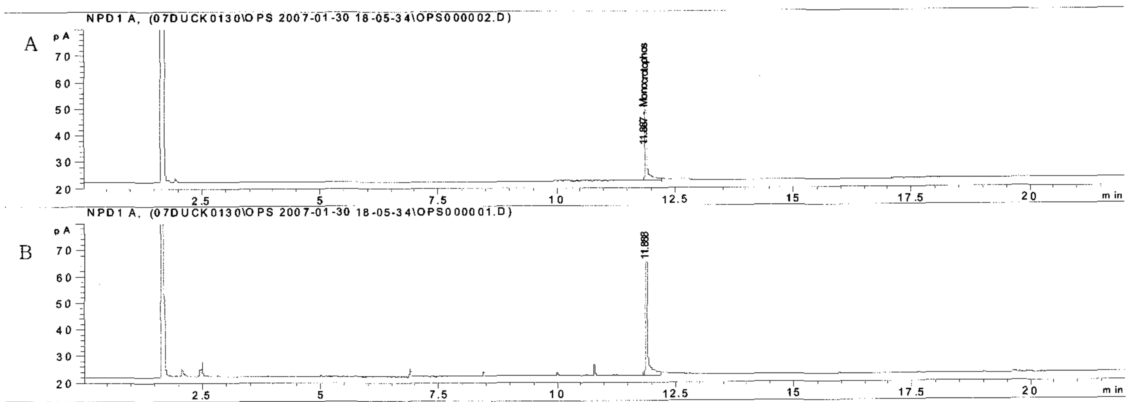

Fig 2. Chromatograms of GC/NPD (A : standard , B : sample,)

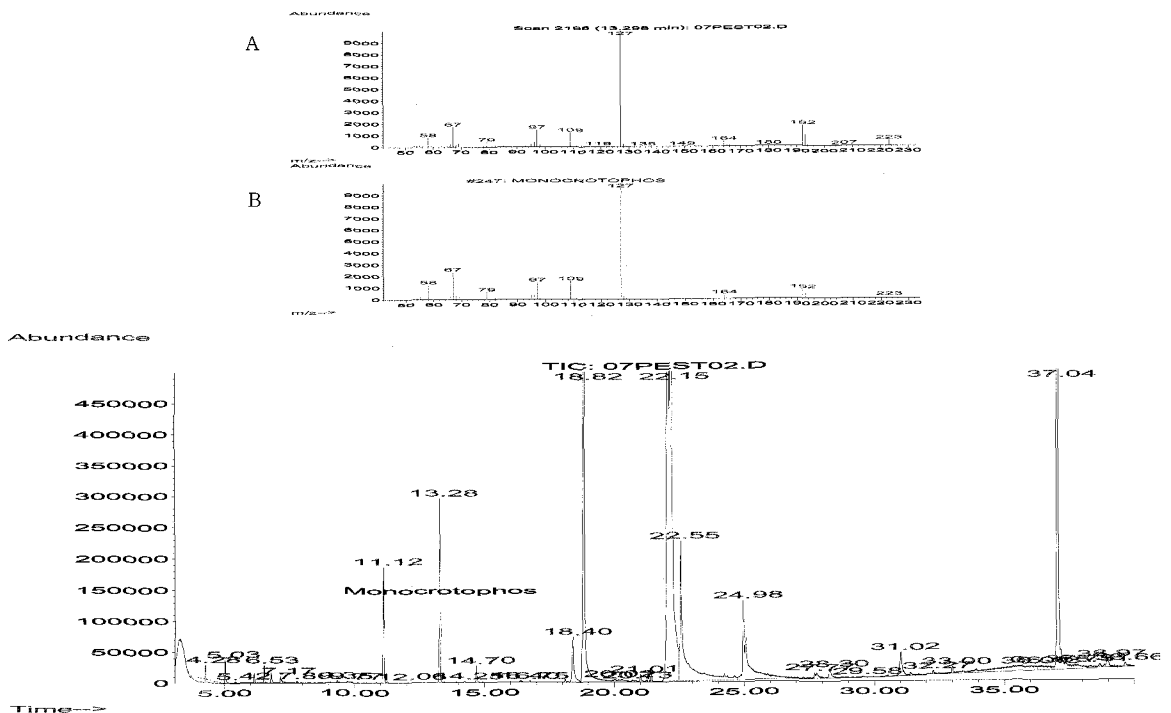

Fig 3. Chromatograms of GC/MSD (A:Nist library searching spectrum , B: sample)

that of NIST library (Fig 3).

Monocrotophos is quantified from 31.3 ppm to 294.3 ppm in the contents of gastrointestinal tract, but there were no chemicals detected in liver and in kidney.

Microbiological examination and PCR

No germs were isolated from liver and cecum samples of the 6 mallards. There was no reaction about avian influenza virus

in PCR conducted with the chorioallantoic fluid after incubating of embryonated SPF egg with lung, and cecum sample (Fig 4).

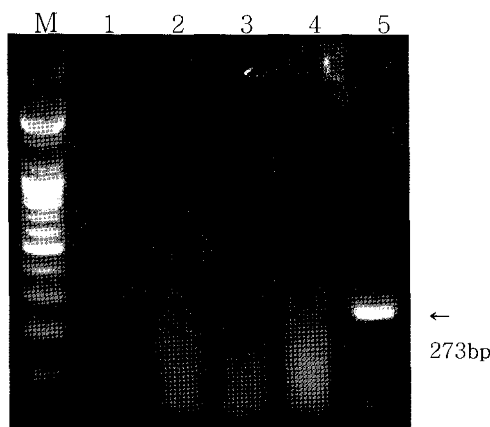

Fig 4. Detection RT PCR assay of avian influenza virus.
M ; 100bp marker, line1-4 : chorioallantoic fluid extracted from embryonated egg with lung, liver, feces and cecum in sample. line 5 : chorioallantoic fluid infected with AI virus.

Discussion

The organophosphoric agricultural chemicals are widely used to get rid of vermins in agriculture industry including livestock and forestry. For example, it is used to control a variety of sucking, chewing and boring insects and spider mites on cotton, sugarcane, peanuts, ornamentals, and tobacco [6]. It can cause severe toxicosis in livestock and wild animals[4] because it is extremely toxic to birds and mammals [7].

Monocrotophos is an organophosphorus insecticide and acaricide which may be affected systemically.

Symptoms of monocrotophos poisoning are similar to those of other organophosphate compounds. Its cholinesterase inhibiting activity causes nervous system effects. Cases of human poisoning are characterized by muscular weakness, blurred vision, profuse perspiration, confusion, vomiting, pain, and small pupils. There is a risk of death due to respiratory failure [8].

Monocrotophos is highly toxic to birds. The LD50 is 0.76mg/kg for California quail, 0.94 mg/kg for bobwhite quail, 1.58mg/kg for Canada goose, 3.3mg/kg for European starling and 4.76mg/kg for mallard ducks [7].

The birds intoxicated with the insecticide show convulsion, incoordination just before death. If the symptoms were developed in the field, however, it is difficult to find and there is no lesion in histopathologic and gross inspection[9, 10].

It is difficult to determine organophosphrus insecticide toxicosis because the dead samples found in the field were usually no symptoms in histopathologic examination. Commonly, the samples with toxicosis have paddies in the gatrointestinal tract [11,12]. Lot of paddies in the mallard crop was observed in this study.

Usually it is not easy to confirm the toxicosis because there were very few symptoms hinting the cause of death at autopsy and the chemicals were decomposed promptly.

To overcome the weak points like these rapid transfer after finding, cold storage of samples, history-taking and listening closely the situation of death are very important.

To verify the death cause of the mallards wintering in Ansung, we had practiced some examinations such as histopathological observation, microbiological test, PCR and chemical analysis, and we can confirm the toxicosis of organophosphoric chemicals as the reason of the death.

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