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Case Report / *Olgu Sunumu*

Acute fipronil intoxication in Squirrel Monkey *(Saimiri sciureus)*

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Abstract: A biocidal product in gel form containing 0.05% fipronil active substance was used for pest control in the habitat of thirteen Squirrel Monkey (*Saimiri sciureus*) in a zoo. Following the application, sudden death was observed in three primates. Routine bacteriological, histopathological and toxicological analyses on one cadaver and tissue samples from the cadaver did not produce any pathogenic agent, pesticide and any pathognomonic findings. In an attempt to investigate the presence of known fipronil intoxication biomarkers, fipronil and fipronil sulfone, we applied modified QuEChERS extraction processes on tissue samples and determined the fipronil and fipronil sulfone levels as 27.1-3.5 µg/kg in cutaneous tissue and 13.8-47.2 µg/kg in brain tissue, respectively, by using UHPLC Q-Orbitrap Mass Spectrometer. In the light of the anamnesis, necropsy, microbiology and histopathology results, and after evaluating the presence of fipronil-fipronil sulfone in tissues, we considered that the deaths could have resulted from acute fipronil toxicity due to species-specific active substance susceptibility.
Keywords: Biocidal, fipronil, fipronil sulfone, intoxication, primate.

Sincap Maymununda (*Saimiri sciureus*) akut fipronil toksikasyonu

Özet: Bir hayvanat bahçesinde barındırılan on üç Sincap maymununun (*Saimiri sciureus*) yaşam alanında haşere mücadelesi amaçlı %0,05 fipronil etkin maddesi içeren jel formunda biyosidal ürün kullanılmıştır. Uygulamanın ardından üç primatta ani ölüm şekillenmiştir. Ölen primatlardan bir tanesinin kadavrasında yapılan nekropsi ve akabinde alınan doku numunelerinde rutin bakteriyolojik, histopatolojik, toksikolojik analizler sonucu patojen etken, patognomik bulgu ve pestisite rastlanılmamıştır. Fipronil toksikasyonu biyobelirteçleri olarak bilinen fipronil ve fipronil sülfon maddelerinin varlığının araştırılması için alınan doku numunelerine modifiye QuEChERS ekstraksiyon işlemleri uygulanmış ve UHPLC Q-Orbitrap Kütle Spektrometresi ile fipronil ve fipronil sülfon sırasıyla deri dokusunda 27,1-3,5 µg/kg ve beyin dokusunda 13,8-47,2 µg/kg düzeyinde tespit edilmiştir. Anamnez, nekropsi, mikrobiyoloji ve histopatoloji bulguları ışığında, doku fipronil-fipronil sülfon varlığı değerlendirildiğinde türe özgü etkin madde hassasiyeti olabileceği düşünülerek akut fipronil toksikasyonu sonucu ölüm şekillendiği kanaatine varılmıştır.
Anahtar sözcükler: Biyosidal, fipronil, fipronil sülfon, toksikasyon, primat.

Fipronil is a phenylpyrazole derivative broad-spectrum insecticide, which has been used in pets, households, and farming areas as an active substance for the control of target pests since 1993 (15). Upon considering the exposure of other non-target organisms, it was determined that intoxication occurred in humans generally through splash and spills during the use of fipronil products for their pets, or during their stay in houses with insufficient ventilation after fipronil use (14). It was reported that environmental contamination levels caused by fipronil use have reached concentration levels that can pose extensive negative biological and ecological effects on such non-target species as harmless terrestrial and aquatic invertebrates (e.g. bees and worms), which play fundamental roles in ensuring the functioning of the ecosystem (18). Taking into consideration the common use and the environmental life cycle of the drug, investigating its non-target toxicity effects on ecosystems and food chain (apart from the targeted efficacy on pests) is a remarkable current topic for the one health concept.

A day after the application of a gel biocidal product containing 0.05% fipronil for pest control, three animals

were found dead in a cage containing 13 Squirrel Monkey (*Saimiri sciureus*) animals, homeland of which was South America. Furthermore, one animal had a number of symptoms of depression, inappetence, lethargy and body weight loss, which have reduced and disappeared over time, and the animal recovered in time. To investigate the cause of death, the cadavers of five elderly male animals were brought to Pendik Veterinary Control Institute. The cadavers were examined by using the primate necropsy techniques (4), samples were collected in line with the routine protocols followed in sudden death cases, and cultured in Blood Agar and MacConkey Agar for isolation and identification under the scope of microbiological controls. No growth was observed at the end of the incubation period. For histopathology examination, tissue samples were stained with Hematoxylin-Eosin (HE) and examined under a light microscope. Histopathology examination revealed parenchymatous degeneration in heart muscle, emphysema, edema, thrombosis, and fibrin matrix in lungs, and hemorrhage and necrosis areas in spaces of Disse in the liver. Extraction was performed with Quechers extraction kits (Agilent Tech., cat. no. QP6150S) for toxicological examination, and no detectable levels of pesticides were determined in the cadaver brain, liver and skin tissue samples by Gas chromatography - Mass Spectrometry (GC-MS) in terms of the pesticide groups of organic phosphorus, organochlorides, carbamates and pyrethroids. Since no other macroscopic pathological results were observed in the cadaver apart from the generalized cachexia that was in line with fipronil intoxication results in the literature, it was decided to perform chemical examination on tissues for fipronil and its metabolites/degradants (mainly fipronil sulfone), which are known to be residual biomarkers of fipronil exposure, in order to confirm fipronil intoxication (8). The presence of fipronil - fipronil sulfone was investigated in cadaver brain, liver and skin tissue samples against the skin, brain and liver tissue samples taken from a six-month-old male Balb/c mouse, which was known to be blank (as per the permission by Pendik Veterinary Control Institute Animal Experiments Local Ethics Committee 10/2019-220). The samples were analyzed with some changes on the method (5) in the literature and by using the Q Exactive High Performance Quadrupole Orbitrap Mass Spectrometer.

Fipronil reference standard (120068-37-3 cas no, Dr. Ehrenstorfer) and fipronil sulfone reference standard (120068-36-2 cas no, Sigma-Aldrich) were prepared for working standards at concentration of 5 µg / mL with acetonitrile (Sigma, LC MS grade). 2.5 grams brain, liver and skin tissue samples weighed for the blank and cadaver. Concentration of 5 µg/mL fipronil and fipronil sulfone working standard were loaded in blank mouse skin, brain, liver tissue with 50, 100 and 150 µL spikes. Then, 5 ml of pure water was added to all blank and cadaver samples. After mixing for 45 minutes at low speed, it was kept in an ultrasonic bath for 45 minutes. After the ultrasonic bath, 10 ml of acetonitrile was added to the sample, and vortexing was performed for 5 minutes. Then, 3 grams of $MgSO_4$, 1 gram of NaCl and 2 grams of sodium acetate were added to the sample, and vortexing was performed for 5 minutes. After vortexing, centrifugation was performed at 4500 rpm for 22 minutes at 22 degrees Celsius, the lower phase was taken with a sterile injector and passed through a 0.45 µm filter to the vial.

The prepared samples were delivered to the Q Exactive High-performance Quadrupole Orbitrap Mass Spectrometer. Thermo Scientific Accucore Phenyl Hexyl 100 x 2.1 column was used for analysis. For the flow to be used in the analysis, Ultra-Pure Water containing 0.1% formic acid-2 mM Ammonium Format on line A and Methanol solution containing 0.1% formic acid- 2 mM Ammonium Format on line B were used. The created instrumental method was determined as 15 minutes. The flow gradient was set at 90% from line A, 50% at 3 min, from 100% B line at 5 min to 9 min, and at 0.3 ml / min from 90% A line at 9 min to 15 min. Fipronil and fipronil sulfones were determined in the UHPLC Q-Orbitrap system by making 5 µL injection from the sample that was extracted into the vial prepared for analysis.

Fipronil and fipronil sulfone was determined to be 27.1-3.5 µg/kg in monkey skin tissue and 13.8-47.2 µg/kg in monkey brain tissue, respectively. No detectable levels of fipronil and fipronil sulfone were found in the cadaveric liver tissues.

In light of medical history, necropsy, microbiology, and histopathology results and after considering the presence of fipronil - fipronil sulfone in tissues, it was concluded that the reason of death was acute fipronil intoxication due to possible species-specific active substance susceptibility.

Preparations of fipronil are commonly used in veterinary treatment for ectoparasitary infestations of cats and dogs such as lice, flea, and ticks in spot-on and spray form for external use. Since it is not approved in other animal species such as primates, there are dose and administration recommendations on the literature for its extra label use upon need (9). Fipronil prevents the flow of chloride ions in chloride channels bound to the receptors of gamma-aminobutyric acid (GABA). It leads to excessive nerve activation, and hyperexcitation and death in target species with its GABAergic antagonistic effect formed through nervous system's primary inhibitor neurotransmitter, GABA. The main metabolite in mammals is fipronil sulfone in liver, adipose tissue and urine, and this metabolite is formed by cytochrome P450 in humans (15). Fipronil degrades in the environment through reduction, hydrolysis, oxidation and photolysis

reactions and rapidly forms five main active metabolites (fipronil-sulphate, fipronil-amide, fipronil sulfone, fipronil-desulfinyl, fipronil sulfonic acid) (7). According to the route of administration, technical fipronil (97% purity) is classified in toxicity category II (moderately toxic) or toxicity category III (slightly toxic) (15). Due to the differences in the affinity of main compound fipronil against receptors, while it was reported to be relatively safer in mammals since it showed 500-fold selective toxicity compared to insects (8), it has been reported that the primary biological metabolite of fipronil, fipronil sulfone is 20 times more active in mammalian chloride channels compared to chloride channels of insects, and that primary environmental metabolite fipronil desulfinyl was 9-10 times more active in mammalian chloride channels compared to the main compound (3). Upon examining the species that had been exposed to fipronil, its metabolites and environmental effects, it was reported that no gender difference was determined in the toxicokinetics or metabolism despite significant interspecies differences (2). Although fipronil is reported to be highly toxic for a number of bird species (e.g. chicken), it is not toxic against others (e.g. duck) (12), and it was also reported upon examining fipronil and fipronil-sulfone distribution and bioaccumulation in susceptible birds exposed to fipronil that it passed the blood-brain barrier, sulfone formed significantly high tissue concentrations in brain, liver and adipose tissue, and that the elevation in brain sulfone levels being simultaneous with specific intoxication effects provided insight for possible toxicity mechanisms in susceptible species (11). Placental passage or passage through milk to offspring was reported in mother rats exposed to fipronil and it resulted in neurotoxic effects in central nervous system including modified memory behaviour (16), and it was also reported that fipronil and its active metabolite sulfone caused reduction in motor coordination (17). Results on humans have determined that fipronil sulfone in maternal serum passed through from placenta to the fetus and formed negative side effects for infants (10). A series of adverse reaction reports of anorexia, lethargy, contractions, and death have been reported after the off-label use of fipronil in young and juvenile rabbits (19). Moreover, very small doses of fipronil applied to control grasshoppers have been reported to show negative effects on non-target species such as Coleoptera, Hymenoptera and Diptera, which are important for agriculture (7, 18). A pretty large part of pesticides in the market is used in agricultural areas. Particularly in vertebrate populations exposed to metabolites of environmental pesticide cycle, negative effects may be observed directly through intoxication or indirectly through the effects on growth, development, and reproduction due to the decrease in food sources (6, 13). The homeland and living area of Squirrel Monkey that was addressed in this case report is largely limited to Amazon River Delta within the borders of Brazil (1). It has been reported that Brazil is among the largest producers and exporters of agricultural products, and accordingly one of the largest pesticide consumers around the world, and that 1.068.60 tons of fipronil was used in Brazil for agricultural purposes in 2012 (15). It is known that fipronil metabolites form a significant effect on mammalian receptors, and through exposure to these metabolites, it is known that selectivity among receptors of non-target species such as insects and humans is decreased compared to the main compound. This case was shared particularly to emphasize that these metabolites in the environmental pesticide cycle may lead to larger problems for non-target species in the ecosystem such as the Squirrel Monkey, and to form an important step in the investigation of the environmental pesticide exposure. In the future, there is a need for detailed studies to be performed on susceptible species and their living areas in order to reveal fipronil exposure status and possible toxicity mechanisms.

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Ethical Statement

This study was approved by the Pendik Veterinary Control Institute Animal Experiments Local Ethics Committee (10/2019-220).

Conflict of Interest

The authors declared that there is no conflict of interest.

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