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Case Report

Post-Mortem Analysis of Diazinon and its major Metabolite, 2-isopropyl-4-methyl-6-hydroxypyrimidine, in a Case of Fatal Diazinon Ingestion

تحليل ما بعد الوفاة لمادة الديازينون Diazinon وناتج الأيض الرئيسي 2-isopropyl-4-methyl-6-hydroxypyrimidine في حالة وفاة نتيجة تناول جرعة قاتلة من الديازينون

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Abstract

This case report describes a detection and quantitation method for diazinon and its major metabolite, 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP), in postmortem blood and tissue samples from a fatal case of diazinon ingestion. Diazinon and IMHP were extracted from postmortem samples with a liquid/liquid method and analyzed by gas chromatography mass spectrometry (GC/MS).

By comparing to diazinon standard and matching the retention time, diazinon was detected in two visceral organs, the stomach (0.89 μg/g) and the small intestine (8.80 μg/g). The highest level of diazinon was detected in the small intestine (8.80 μg/g), whereas the highest amount of IMHP was noted in the kidney (0.84 Area %) and bladder (0.75 Area %).

In conclusion, determination of IMHP in postmortem samples could be used as an indicator for diazinon exposure, especially in the case of delayed death; whereas, the small intestine could be the best source of sample in diazinon assessment in cases of fatal diazinon ingestion.

Keywords: *Forensic Sciences, Diazinon, IMHP, Pesticides, GCMS, Postmortem, Forensic Toxicology.*

المستخلص

من خلال تقرير الحالة المدروس تم وصف طريقة كشف وتحديد كمي لمادة الديازينون وناتج أيضه الرئيسي 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP-6) في عينات الدم والأنسجة ما بعد الوفاة لحالة وفاة ناتجة عن تناول مادة الديازينون. واستخلصت مادة الديازينون Diazinon ومادة الـ IMHP من عينات ما بعد الوفاة بطريقة استخلاص سائل - سائل ومن ثم حللت بواسطة جهاز كروماتوغرافيا الغاز المقترن بمطياف الكتلة (GC/MS). وأظهرت النتائج وجود الديازينون في كل من عينات المعدة (0.89 μg/g) وعينات الأمعاء الدقيقة (8.80 μg/g) وذلك من خلال مقارنتها بمادة الديازينون القياسية ومطابقة زمن الاحتفاظ. وتم الكشف عن أعلى مستويات الديازينون في الأمعاء الدقيقة (8.80 μg/g)، في حين وجدت أعلى كمية من IMHP في عينات الكلى (0.84 مساحة سطحية٪) وعينات المثانة (0.75 مساحة سطحية ٪). وتستنتج الدراسة أن تحديد مادة الـ IMHP في عينات بعد الوفاة يمكن أن يستخدم كدليل إرشادي للتعرض لمادة الديازينون خاصة في حالة الوفاة المتأخرة، في حين أن الأمعاء الدقيقة يمكن أن تكون أفضل عينة في تقييم الديازينون في حالة ابتلاع الديازينون المميت.

الكلمات المفتاحية: علوم الأدلة الجنائية، IMHP، Diazinon، المبيدات، GC/MS، ما بعد الوفاة، علم السموم الجنائي.

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1. Introduction

Diazinon is a regularly utilized organophosphorus pesticide in agricultural settings, and classified as a class II hazardous pesticide by the WHO (Class II: moderately hazardous pesticide). It is also among the most common causes of pesticide poisoning in several countries [1]. It has been broadly utilized as a part of some household pesticides [2]. As a result of its risk to human health, the domestic use of diazinon was banned in the United States and Canada [3, 4]. In numerous countries, diazinon is still commonly used for household pesticide purposes and misused for the treatment of head lice [5, 6]. Since diazinon is still utilized for residential use to control a variety of household insects in Saudi Arabia, diazinon exposure and toxicity in the general population, especially women and children, are expected to be high. Diazinon toxicity may occur through lungs and skin, but this usually depends on the purpose and method of its application. It has a rapid and extensive oral absorption and can be absorbed through the lung and skin. Its absorption depends on the applied area and the type of vehicle used [7, 8].

There is a limited data regarding the distribution of diazinon in the human body. In a fatal case of diazinon ingestion, diazinon was detected in several tissues in postmortem samples [7, 8]. Some fatality cases showed that the victims died with acute pancreatitis, cardiac arrest, respiratory failure and coma [9, 10]. However, the main cause of death was mainly attributed to respiratory failure and cardiac arrest [11]. A diazinon-related death report showed petechial hemorrhages in the stomach and gastric mucosa during autopsy of a 54-year-old female who ingested around 293 mg/kg of diazinon [7]. Another diazinon related death showed a cardiac and respiratory arrest in an 8 year-old girl who accidently ingested a mixture of diazinon and parathion pesticide [12]. The toxic effects may be attributed to the inhibition of acetylcholinesterase (AChE) by diazinon and its metabolites [9]. From a toxicokinetic point of view, diazinon is highly and rapidly metabolized to 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP). Due to the rapid metabolism, diazinon level is useful to evaluate recency of exposure, while IMHP is commonly used as an indicative marker for diazinon exposure [13].

A report from the Department of Preventive Medicine in the General Directorate of Health Affairs, Jazan (KSA) showed that diazinon cases were the most frequent cases of organophosphorus poisoning and that it has been associated with stomach hemorrhages, cardiac arrest and respiratory arrest (unpublished data). In this report, a fatal case of diazinon ingestion and methods ot its extraction and detection are presented.

2. The Case

A 30-year-old male ingested an unknown amount of diazinon (Diazinon 60%). He was admitted to the hospital twelve hours prior to his death. During hospitalization, pralidoxime and atropine doses were given and gastric lavage was performed to prevent further absorption of the ingested diazinon in the stomach. After death, postmortem samples were sent for toxicological analysis as organophosphorus suspected death. The goal of this report was to discuss an analytical technique to determine the presence of diazinon and IMHP in postmortem samples from a fatal case of diazinon ingestion. The method of extracting and analyzing diazinon and its metabolites are discussed.

3. Materials and Methods

3.1. Reagents and Chemicals

Petroleum ether, diethyl ether and ethyl acetate were

obtained from Sigma-Aldrich (Sigma Aldrich, Germany). Diazinon standard was supplied by King Faisal Specialist Hospital and Research Centre (KFSH&RC, Saudi Arabia).

3.2. Sample Preparation

All samples were extracted without pH control by the liquid-liquid extraction method described by Kiljanek et al. (2011) with some modifications [14]. Briefly, 1 mL of blood sample or 1 gram of small segments of each tissue sample (brain, liver, kidney, stomach, bladder and small intestine) were placed in a 50 mL glass vial with a screw cap. Five mL of organic solvent (mixture of petroleum ether and diethyl ether 4:1) was added to each sample and allowed to stand for 24 hours at room temperature. The next day, vials were vortexed and centrifuged for 5 minutes at 3000 RPM. Then, 3.5 mL of organic layer in each vial was filtered and transferred to clean 16 ml glass tubes and evaporated to dryness under nitrogen stream. The residues were reconstituted with 75 μL ethyl acetate and made ready for GCMS analysis. Matrix matched calibrators were prepared by spiking negative samples with diazinon standard. Calibration levels were 100, 200, 400, 600, 800, 1000 and 1500 ng/ml (Figure-1). Control samples were prepared by spiking diazinon standard to negative samples of various matrices, and the levels were 100, 400 and 1000 ng/mL. Both calibrators and controls were extracted using the same extraction method.

3.3. GC-MS Analysis

Samples were analyzed using Shimadzu GC/MS instrument (GCMS-QP2010 ultra). An injection volume of 2 μL of each sample was utilized and the GC/MS was set to 270 ºC in splitless mode. Helium was the carrier gas set at a flow rate of 1.2 mL/min. The chromatographic separa-

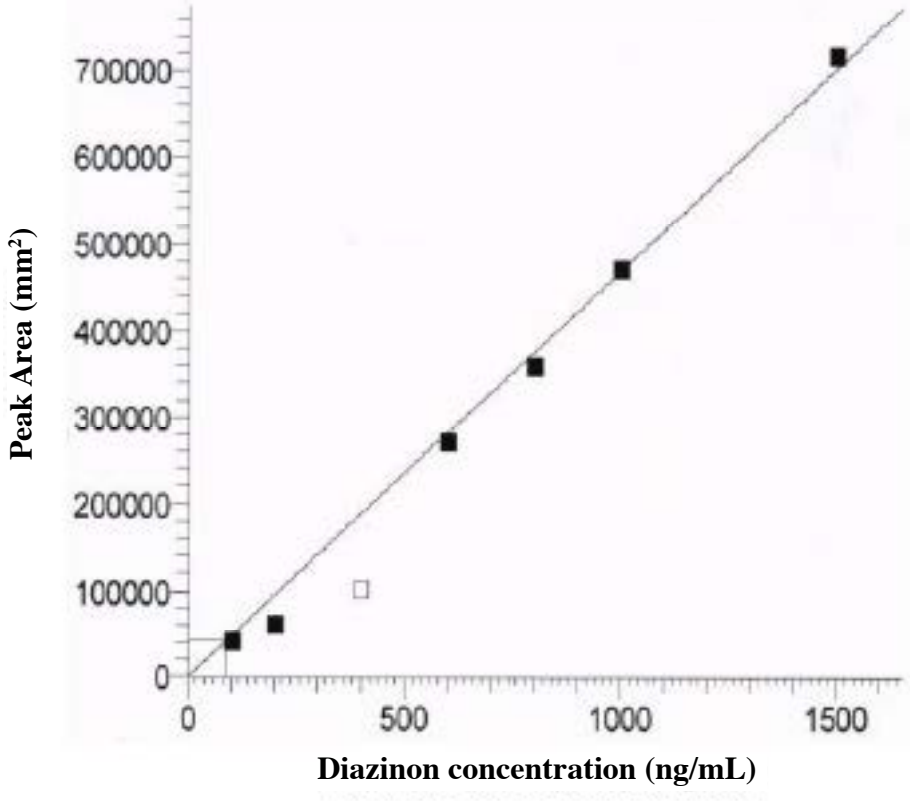

Figure 1- *Calibration curve for diazinon (100, 200, 400, 600, 800, 1000 and 1500 ng/mL) by GC/MS, R^2=0.995.*

tion was carried out using Restek capillary column (Rxi®-5Sil MS) with 30 meter length, 0.25 mm ID and 0.25 μm film thickness. The initial temperature was 70 ºC and was ramped to 290 ºC at a rate of 15 ºC and held for 20 minutes at the maximum temperature. The transfer line temperature was 240 ºC and the EI ion source was set at 200 ºC. The total time of GC/MS analysis was 30 minutes for each sample. The MS was operated in scanning mode, starting from m/z 40 to m/z 500.

4. Results

GC/MS analysis of samples showed that the parent compound (diazinon) was detected only in two visceral organs, the stomach (0.89 μg/g) and the small intestine (8.80 μg/g). GC/MS chromatogram acquired for small intestine (Figure-2) showed diazinon peak at a retention time (RT) of 9.81 min and m/z 179, 137 and 152 in the spectrum. Diazinon detection was confirmed by using a diazinon standard and matching the retention time and library search between the samples and the standard. The retention time of the diazinon standard was 9.81 min. Also, all samples (brain, liver, kidney, stomach, blood, bladder and small intestine) were positive for diazinon major metabolite (2-isopropyl-4-methyl-6-hydroxypyrimidine; IMHP) as the library search revealed scores above 90% with similarity index (SI). The GC/MS chromatogram (Figure- 3) showed IMHP peak at a RT of 6.37 min and m/z 137, 152 and 84 in the spectrum. Table-1 shows analysis of postmortem samples.

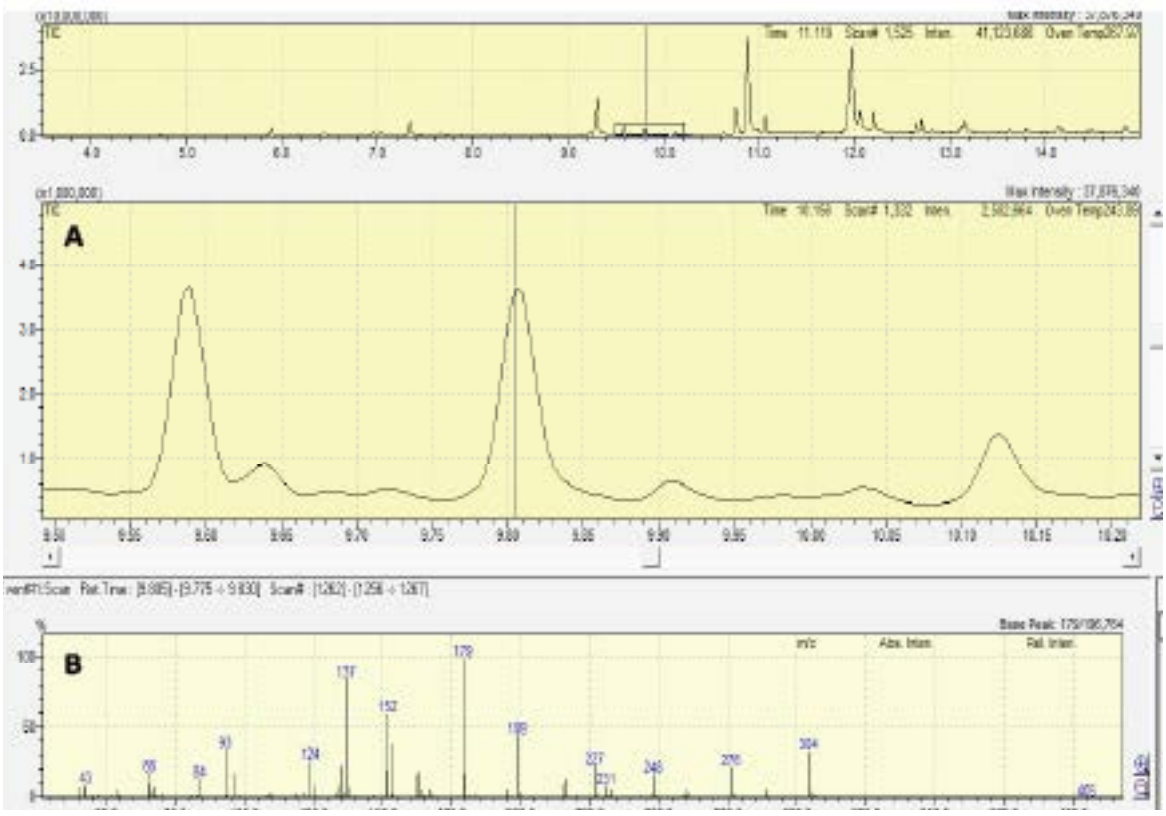

Figure 2- *GC-MS chromatogram of extracted diazinon in small intestine. Window (A) shows the RT at 9.81 min. Window (B) shows the acquired spectrum with the three main fragments at m/z 179, 137 and 152.*

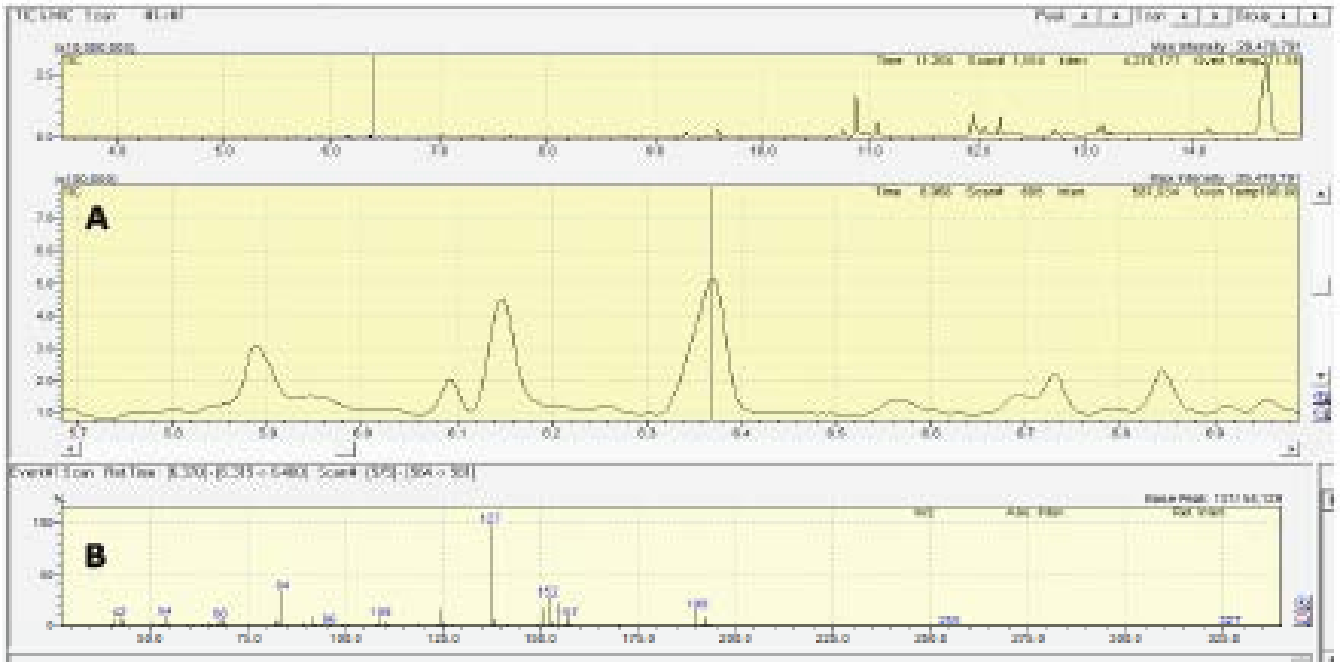

Figure 3- *GC-MS chromatogram of diazinon metabolite IMHP in Liver. Window (A) shows the RT at 6.37 min. Window (B) shows the acquired spectrum with the three main fragments at m/z 137, 152 and 84.*

Table-1: *GC-MS analysis of diazinon and IMHP in postmortem samples.*

Sample Type	Diazinon				2-Isopropyl-6-methy-4-pyrimidone			
	µg/g	Rt (Min)	M/Z	Detected	Rt (Min)	M/Z	SI %	Area %
Brain	ND	-	-	Yes	6.38	137,152,84	93	0.74
Liver	ND	-	-	Yes	6.37	137,152,84	89	0.29
Kidney	ND	-	-	Yes	6.38	137,152,84	93	0.84
Stomach	0.89	9.82	179,137,152	Yes	6.38	137,152,84	94	0.36
Bladder	ND	-	-	Yes	6.37	137,152,84	86	0.75
Blood	ND	-	-	Yes	6.36	137,152,84	73	0.59
Small Intestine	8.80	9.81	179,137,152	Yes	6.43	137,152,84	94	0.55

ND, Not Detected; Rt, Retention Time; SI, Similarity Index; Area %, The percentage of peak area, which is proportional to the amount of diazinon

5. Discussion

The residential use of diazinon still occurs in the Jazan region, Saudi Arabia. Therefore, the poisoning with diazinon belong to the most frequent cases among organophosphorus toxicities. Women and children were the most prevalent, possibly because women misuse diazinon as a home pesticide in non-ventilated areas as well as a treatment for head lice in children [5]. Most of the diazinon ingestion cases were nonfatal [15, 16], but some rare fatalities have also been reported [7, 12]. Therefore, restricting the use of diazinon and raising awareness in the community about the dangers of diazinon and how to use it safely are necessary preventative measures.

Most signs and symptoms of diazinon toxicity are related to cholinergic manifestation, which is mediated through the inhibition of serum AChE activity. Toxicity increases when the reduction reaches 50% or more in enzyme activity [17]. In the present case, the blood sample was hemolyzed and, therefore, was not eligible for serum AChE activity analysis by a colorimetric method that could be utilized as a confirmatory test for organophosphorus poisoning. Therefore, the detection of diazinon and IMHP in postmortem samples was used as a confirmatory test for diazinon exposure. From the toxicokinetic point of view, diazinon is a basic lipophilic compound with a high volume of distribution that may undergo postmortem redistribution [18, 19].

Diazinon was found in low concentration in the stomach (0.89 μg/g) as compared to small intestine (8.80 μg/g), which could due to the rapid absorption or possibly from the gastric lavage that was performed during hospitalization. The high concentration of diazinon found in the small intestine could be due to the high lipophilic property of diazinon that lead to its accumulation in this site [20]. The negative results for diazinon in other organ extracts could be attributed to the rapid metabolism of diazinon [21]. Such rapid metabolism is mediated by CYP1A1, the primary enzyme involved in diazinon bioactivation, and CYP2C19, the primary enzymes involved in diazinon detoxification [22], which are highly expressed in the human liver. Therefore, determination of the diazinon in stomach and small intestine samples can provide evidence of recent exposures in cases of fatal diazinon ingestion.

In addition, IMHP, the major metabolite of diazinon, was found in all postmortem samples. According to the peak areas of the IMHP, the highest levels were observed in the kidney, then in the bladder and the brain, followed by blood and small intestine. The lowest levels were noted in stomach and liver (Table-1). It is difficult to compare this case with other cases due to different circumstances of death and no data available about the quantity of diazinon consumed. Furthermore, different analytical techniques and instruments are used in the extraction and identification of the poison. This case report demonstrates that the determination of IMHP in postmortem samples could be used as an indicative marker for diazinon exposure, especially in the case of delayed death; whereas, the small intestine could be the best sample in diazinon assessment in cases of fatal diazinon ingestion.

Conflict of Interest

There are no financial or other interests with regard to this manuscript that might be construed as a conflict of interest. All of the authors are aware of and agree to the content of the manuscript and their being listed as an author on the manuscript.

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Nill.

References

1. WHO. The World Health Organization recommended classification of pesticides by hazard and guidelines to classification 2009. 2010; Available from: http://apps.who.int/iris/bitstream/10665/44271/1/9789241547963_eng.pdf.
2. Morgan MK, Stout DM, Jones PA, Barr DB. An observational study of the potential for human exposures to pet-borne diazinon residues following lawn applications. Environ res. 2008;107(3):336-42. https://doi.org/10.1016/j.envres.2008.03.004
3. Carlton EJ, Moats HL, Feinberg M, Shepard P, Garfinkel R, Whyatt R, Evans D. Pesticide sales in low-income, minority neighborhoods. J Community Health. 2004;29(3):231-44. https://doi.org/10.1023/B:JOHE.0000022029.88626.f4
4. Kendall P. Public Health Considerations on Cosmetic Use of Pesticides in BC 2013. Available from: http://www.bccdc.ca/resource-gallery/Documents/Guidelines%20and%20Forms/Guidelines%20and%20Manuals/Health-Environment/CosmeticPesticidesinBCweb-final1.pdf.
5. Hamad MH, Adeel AA, Alhaboob AA, Ashri AM, Salih MA. Acute poisoning in a child following topical treatment of head lice (pediculosis capitis) with an organophosphate pesticide. Sudan J Paediatr. 2016;16(1):63.
6. Halle A, Sloas DD. Percutaneous organophosphate poisoning. South Med J. 1987;80(9):1179-81. https://doi.org/10.1097/00007611-198708090-00025
7. Poklis A, Kutz FW, Sperling JF, Morgan DP. A fatal diazinon poisoning. Forensic Sci Int. 1980;15(2):135-40. https://doi.org/10.1016/0379-0738(80)90152-8
8. Garfitt SJ, Jones K, Mason HJ, Cocker J. Exposure to the organophosphate diazinon: data from a human volunteer study with oral and dermal doses. Toxicol Lett. 2002;134(1-3):105-13. https://doi.org/10.1016/S0378-4274(02)00178-9
9. Banerjee I, Tripathi S, Roy AS. Clinico-epidemiological characteristics of patients presenting with organophosphorus poisoning. N Am J Med Sci. 2012;4(3):147-50. https://doi.org/10.4103/1947-2714.93884
10. Yurumez Y, Durukan P, Yavuz Y, Ikizceli I, Avsarogullari L, Ozkan S, et al. Acute organophosphate poisoning in university hospital emergency room patients. Intern Med. 2007;46(13):965-9. https://doi.org/10.2169/internalmedicine.46.6304
11. Harris LW, Fleisher JH, Innerebner TA, Cliff WJ, Sim VM. The effects of atropine-oxime therapy on cholinesterase activity and the survival of animals poisoned with O,O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate. Toxicol Appl Pharmacol. 1969;15:216-24. https://doi.org/10.1016/0041-008X(69)90150-1
12. DePalma AE, Kwalick DS, Zukerberg N. Pesticide poisoning in children. JAMA. 1970;211(12):1979-81. https://doi.org/10.1001/jama.1970.03170120025004
13. Morgan MK, Sheldon LS, Jones PA, Croghan CW, Chuang JC, Wilson NK. The reliability of using urinary biomarkers to estimate children's exposures to chlorpyrifos and diazinon. J Expo Sci Environ Epidemiol. 2011;21(3):280-90. https://doi.org/10.1038/jes.2010.11
14. Kiljanek T, Niewiadowska A, SEMENIUK S. Determination of organophosphorus pesticide residues in the liver by gas chromatography with flame photometric detection. Bull Vet Inst Pulawy. 2011;55:731-6.
15. Dulaurent S, Gaulier JM, Blanc-Lapierre A, Im-

bert L, Lachatre G. Urinary determination of 2-isopropyl-4-methyl-6-hydroxypyrimidine in case of non fatal poisoning with diazinon. Forensic Sci Int. 2013;228(1-3):e20-4. https://doi.org/10.1016/j.forsciint.2013.03.001

16. Klemmer HW, Reichert ER, Yauger WL, Jr. Five cases of intentional ingestion of 25 percent diazinon with treatment and recovery. Clin Toxicol. 1978;12(4):435-44. https://doi.org/10.3109/15563657809150014
17. Mancini F, Jiggins JL, O'Malley M. Reducing the incidence of acute pesticide poisoning by educating farmers on integrated pest management in South India. Int J Occup Environ Health. 2009;15(2):143-51. https://doi.org/10.1179/oeh.2009.15.2.143
18. Tomokuni K, Hasegawa T, Hirai Y, Koga N. The tissue distribution of diazinon and the inhibition of blood cholinesterase activities in rats and mice receiving a single intraperitoneal dose of diazinon. Toxicol. 1985;37(1-2):91-8. https://doi.org/10.1016/0300-483X(85)90115-5
19. Wu H, Evreux-Gros C, Descotes J. Diazinon toxicokinetics, tissue distribution and anticholinesterase activity in the rat. Biomed Environ Sci. 1996;9(4):359-69.
20. Lau C, Chung K, Kam C. Accidental organophosphate insecticide poisoning: a fatal child case with hazards to emergency department staff. HONG KONG JEMERG ME. 2003;10(4):248-52. https://doi.org/10.1177/102490790301000407
21. Iverson F, Grant D, Lacroix J. Diazinon metabolism in the dog. Bull Environ Contam Toxicol. 1975;13(5):611-8. https://doi.org/10.1007/BF01685188
22. Ellison CA, Tian Y, Knaak JB, Kostyniak PJ, Olson JR. Human hepatic cytochrome P450-specific metabolism of the organophosphorus pesticides methyl parathion and diazinon. Drug Metab Dispos. 2011:dmd.111.042572.