QUANTIFYING ACETYLCHOLINESTERASE INHIBITION: A DIAGNOSTIC TOOL FOR ORGANOPHOSPHATE POISONING

Andreea-Camelia HÎRJĂU [12*], Ilinca-Mihaela MARANDIUC [2], Gabriel-Lucian RADU [1]

This study investigated acetylcholinesterase (AChE) inhibition and recovery dynamics in seven patients with acute diazinon (n=4) or malathion (n=3) poisoning. AChE activity, measured serially, showed significant inhibition on admission, correlating strongly with insecticide concentration (diazinon: r=-0.95; malathion: r=-0.99). Diazinon cases exhibited faster and more consistent AChE recovery than malathion. The findings suggest that AChE monitoring is valuable for assessing OP poisoning severity and progression, but insecticide-specific recovery patterns should be considered when interpreting AChE trends and guiding treatment decisions.

Keywords: Organophosphate poisoning; Acetylcholinesterase (AChE); Diazinon; Malathion; Recovery dynamics; GC-MS/MS.

1. Introduction

Organophosphate (OP) compounds, such as diazinon and malathion, are widely employed insecticides in agriculture and pest control. However, these chemicals pose a significant risk to human health due to their potential for accidental or intentional poisoning [1][2]. OP intoxications remain a global health concern, particularly in rural communities and developing countries where access to these substances may be less regulated [3 – 6].

OPs exert their toxicity primarily through the inhibition of cholinesterase, a vital enzyme responsible for the hydrolysis of the neurotransmitter acetylcholine (AChE) [7][8]. This inhibition disrupts nerve impulse transmission, leading to the accumulation of acetylcholine at cholinergic synapses and excessive stimulation of cholinergic receptors. The clinical presentation of OP intoxication is dominated by a cholinergic crisis, characterized by a wide range of symptoms including muscarinic effects (e.g., miosis, bradycardia, bronchospasm, diarrhea), nicotinic

* Corresponding author
[1] Doctoral School of "Chemical Engineering and Biotechnologies", National University of Science and Technology POLITEHNICA Bucharest, Romania, e-mail: varzaru_camelia@yahoo.com; lucian.radu@incdsb.ro
[2] *Cantacuzino* National Institute for Medical-Military Research and Development, Bucharest, Romania, e-mail: hirjau.andreea@cantacuzino.ro; marandiuc.ilinca@cantacuzino.ro;

effects (e.g., muscle fasciculations, weakness, paralysis), and central nervous system manifestations (e.g., seizures, coma) [9]. The severity of symptoms can vary significantly depending on the specific OP compound, the dose and route of exposure, and individual factors [10 – 12]. Scientific and medical literature often presents general percentage ranges of AChE inhibition to aid in assessing intoxication severity. Although providing broad categories of severity (e.g., mild, moderate, severe) based on AChE activity, these classifications often fail to capture the substantial variability observed in clinical practice. Indeed, numerous factors profoundly affect the correlation between AChE inhibition and clinical outcomes, including the specific OP compound, individual patient variability, and, critically, the timing of cholinesterase measurement.

The diagnosis of OP poisoning often relies on a combination of clinical presentation, exposure history, and laboratory investigations. While the characteristic cholinergic toxidrome can provide strong clinical suspicion, laboratory confirmation is crucial for definitive diagnosis and to guide treatment decisions [13]. Measurement of cholinesterase activity, particularly AChE, is a commonly employed laboratory test to assess the degree of OP-induced enzyme inhibition [14]. Traditional methods for measuring cholinesterase activity, such as spectrophotometric and pH titration assays, can be limited by sensitivity and susceptibility to interference [15]. More advanced techniques, such as gas chromatography (GC) and liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS), offer superior selectivity and sensitivity for the detection and quantification of OP pesticides in biological samples [16 – 30]. However, these methods, including GC-MS/MS, require specialized equipment and technical expertise, and may have longer turnaround times compared to rapid screening methods. Potential limitations also include matrix effects, particularly in complex biological samples, although steps such as liquid-liquid extraction and the use of internal standards can mitigate these effects, as detailed in our study.

Despite the known role of cholinesterase inhibition in OP intoxication, studies specifically examining the effects of malathion and diazinon, as well as the dynamics of AChE recovery, remain insufficient. Furthermore, most studies have focused on the acute effects of OP intoxication, with limited attention given to the longitudinal changes in AChE activity during the recovery phase. To address these knowledge gaps, this study investigates the temporal changes in acetylcholinesterase (AChE) activity in patients with acute diazinon and malathion intoxication. We hypothesize that these two OP compounds will exhibit distinct patterns and rates of AChE recovery, reflecting variations in their toxicokinetic and pharmacodynamic profiles. The objectives of this study are to characterize temporal changes in AChE activity, compare the rate and pattern of AChE recovery between diazinon and malathion, and explore the relationship between AChE activity and clinical outcomes. It is anticipated that the findings

will contribute to a more comprehensive understanding of the dynamics of AChE inhibition and recovery in OP intoxication, potentially informing the refinement of diagnostic and therapeutic strategies.

2. Experimental

2.1. Study Design and Patients

This study analyzed blood and urine samples from seven patients admitted to the Intensive Care Unit (ICU) Toxicology Department at Bucharest Emergency Clinical Hospital between 2021 and 2023. The sample size was determined by the number of patients presenting to the ICU with acute diazinon or malathion poisoning during this period. This retrospective study adhered to the principles of the Declaration of Helsinki regarding ethical research. Patient data was collected following a comprehensive review of records, and informed consent was obtained where possible. All patient identifiers were removed to ensure privacy and confidentiality. Initial confirmation of acute organophosphate (OP) intoxication was established through pesticide screening of urine samples collected in sterile containers.

For the urine samples, a 30 mL aliquot was taken and spiked with 50 µL of a 100 µg/L cypermethrin internal standard. Liquid-liquid extraction (LLE) was performed using 15 mL of a 1:1:1 (v/v/v) mixture of chloroform, dichloromethane, and methyl chloride. The mixture was magnetically stirred for 5 minutes and then centrifuged at 5000 RPM for 10 minutes. The organic phase was collected and evaporated to dryness at 100°C. The resulting residue was reconstituted in 1 mL of methanol, and a 1 µL aliquot was subjected to GC-MS/MS analysis.

Subsequently, serial blood samples were collected into vacutainers containing EDTA. All samples were stored at 4°C and processed within one hour of collection. This research was conducted under institutional review board approval and all samples were anonymized.

2.2. GC-MS/MS Analysis for Insecticide Detection

Urine samples were analyzed for diazinon and malathion using gas chromatography-mass spectrometry (GC-MS/MS). This technique separates and quantifies compounds of interest using gas chromatography and mass spectrometry. GC-MS/MS is highly sensitive and specific, providing information about the OP compound involved. GC-MS/MS is well-suited for volatile OP compounds, often found in OP poisoning cases. While HPLC, especially with tandem mass spectrometry (LC-MS/MS), can also be used, it generally requires more extensive sample preparation, particularly for complex matrices like urine. LC-MS/MS offers high sensitivity and specificity, like GC-MS/MS, and is often preferred for non-volatile or thermally labile compounds. However, for volatile

OP compounds in urine, GC-MS/MS often provides a simpler and more robust analytical approach.

Insecticide detection was performed using an Agilent 8890 GC coupled with a 7010B triple quadrupole MS and 7693A Autosampler. Chromatographic separation utilized an HP-5ms column (15 m × 250 µm × 0.25 µm) with helium carrier gas (1.1 mL/min). The GC oven program: 60 °C initial, rapid ramp to 120 °C, and slower ramp to 310 °C.

Analytical conditions for GC-MS/MS included an inlet temperature of 280 °C, splitless injection of 1 µL sample using a wool liner, and an ion source temperature of 300 °C. Electron impact ionization (70 eV) was used, and the mass spectrometer acquired data in full scan mode (40-600 m/z) with a 100-ms scan time.

2.3. AChE Activity Assay

Whole blood acetylcholinesterase (AChE) activity was determined using the Securetec Che Check Mobile System (Detektions-Systeme AG, Germany) via Ellman's method (a spectrophotometric assay that quantifies acetylthiocholine hydrolysis). The reaction produces a yellow product, measured at 412 nm, directly proportional to AChE activity. The Ellman's method is simple and widely used but has limitations, including susceptibility to interference. Assay-specific, pre-filled cuvettes were used. Capillary blood was introduced following a baseline reading. Hemoglobin was measured, then, after cap exchange and substrate dissolution, hemoglobin-corrected AChE activity was measured. Results (U/gHb) were generated within four minutes and did not require calibration. AChE inhibition was calculated against a 49.4 U/gHb reference.

2.4. Statistical Analysis

Statistical analysis was performed using Microsoft Excel. The Pearson correlation coefficient (r) was used to assess the relationship between AChE activity measured by the two methods. A p-value < 0.05 was considered statistically significant.

3. Results and discussion

Seven patients with acute OP intoxication were included in this study. Significant AChE inhibition was observed in all cases, confirming exposure to OP compounds. While both diazinon and malathion induced enzyme inhibition, the degree of inhibition and the subsequent recovery trajectory varied between individuals, suggesting potential inter-individual differences in susceptibility and response to OP toxicity.

Figure 1 illustrates the temporal changes in acetylcholinesterase (AChE) activity (U/gHb) in seven patients admitted with either diazinon or malathion

intoxication. The graph depicts AChE levels measured over a period of up to 24 days of hospitalization. The red dotted line represents the upper limit of the reference interval for AChE (approximately 49 U/gHb), while the yellow dotted line indicates the lower limit of the reference interval (approximately 33 U/gHb).

Data points represent individual AChE measurements for each patient at various time points during their hospital stay. The graph clearly shows an overall upward trend in AChE levels for all cases, indicating recovery of enzyme activity. However, the rate and pattern of recovery vary between cases and between diazinon and malathion poisoning.

Diazinon cases (blue, purple, green, and orange lines) generally demonstrate a more rapid and consistent increase in AChE levels, with most cases reaching or approaching the lower limit of the reference interval within the observation period. In contrast, malathion cases (brown, dark red, and black lines) exhibit a slower and more variable recovery trajectory, with AChE levels remaining below the reference interval for a longer duration.

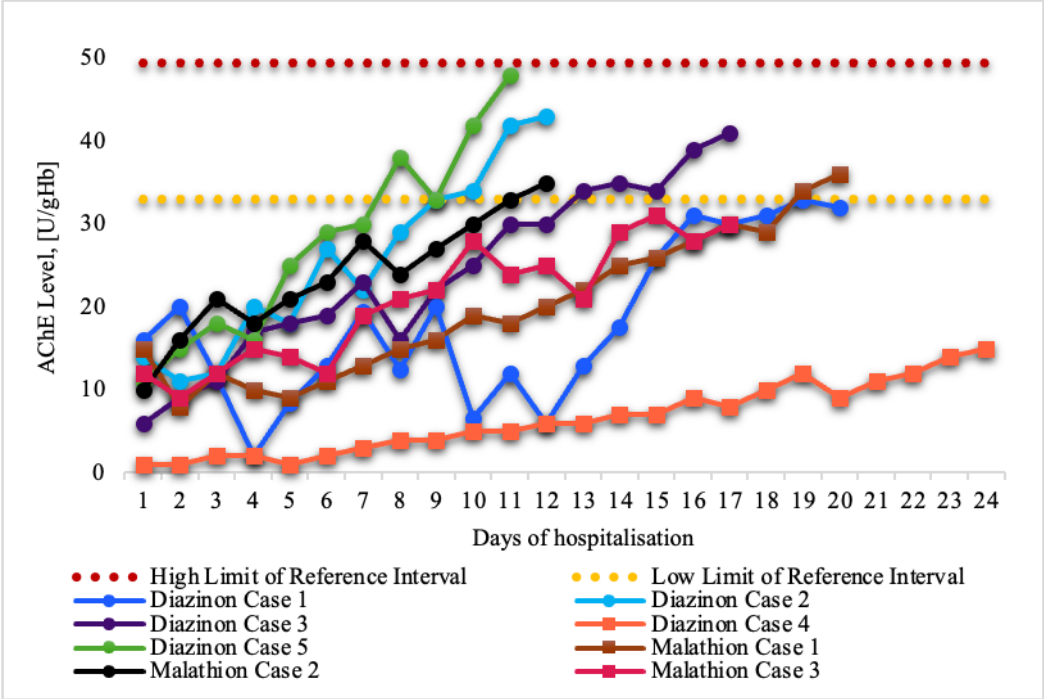

Fig. 1. Acetylcholinesterase (AChE) Recovery Over Time in Organophosphate Poisoning Cases

This graphical representation highlights the dynamic nature of AChE recovery following organophosphate intoxication and suggests potential differences in recovery patterns between diazinon and malathion exposures. As anticipated, initial AChE levels were markedly depressed in all cases, reflecting the inhibitory action of these OP compounds on this critical enzyme. The

subsequent serial measurements revealed a consistent pattern of AChE recovery over time, underscoring the body's capacity to regenerate this enzyme following OP exposure. However, notable variations were observed in the rate and magnitude of recovery across the different cases.

Table 1 presents data from seven patients admitted with acute organophosphate intoxication, categorized by the specific insecticide involved (diazinon or malathion). Key parameters include AChE activity levels at admission and discharge, expressed both in units per gram of hemoglobin (U/gHb) and as a percentage of the upper reference interval (49.4 U/gHb), and the duration of intensive care unit (ICU) stay. As evident from the table, all patients exhibited significantly depressed AChE activity upon admission, indicative of acute OP poisoning. AChE levels at discharge demonstrate substantial recovery in most cases, though the extent varies. Notably, patients with diazinon intoxicaton generally displayed higher AChE recovery percentages at discharge compared to those with malathion poisoning. The duration of ICU stays also varied considerably among patients, with no clear correlation between insecticide type and length of stay.

Table 1

Acetylcholinesterase (AChE) Activity and Clinical Outcomes in Organophosphate Poisoning Cases

No.	Insecticide	Level (Admission), U/gHb	Enzymatic Activity* (Admission), %	Level (Discharge), U/gHb	Enzymatic Activity* (Discharge), %	Days of ICU Admission
1	Diazinon	14	28	43	87	12
2	Diazinon	6	12	41	83	17
3	Diazinon	1	2	15	30	24
4	Diazinon	11	22	48	97	11
5	Malathion	15	30	36	73	20
6	Malathion	10	20	35	71	12
7	Malathion	12	24	30	61	17

Diazinon poisoning cases exhibited a strong, statistically significant positive correlation between time and AChE activity (r: 0.9027-0.9368; $p < 0.001$), suggesting a consistent and predictable recovery. This correlation was calculated using Microsoft Excel software, employing the PEARSON function to determine the relationship between time and AChE activity. The resulting r-values indicate a strong positive correlation, while the p-values ($p < 0.001$) demonstrate that this correlation is statistically significant. This rapid and uniform recovery in diazinon cases may reflect differences in metabolic pathways and toxicokinetic profiles compared to malathion. Conversely, malathion cases showed a less pronounced, though still statistically significant, positive correlation (r: 0.6809-0.8917; $p < 0.0002$), indicating a more variable recovery. These variations likely reflect differences in toxicokinetics, patient metabolism, comorbidities, or initial

intoxication severity. The consistent statistically significant positive correlations across all cases validate serial AChE monitoring as a valuable biomarker for assessing OP intoxication progression and resolution. Distinct trends between diazinon and malathion cases highlight the importance of considering the specific OP compound for accurate recovery prediction and tailored treatment.

Several factors may contribute to the observed inter-individual variability in AChE recovery. The severity of the initial intoxication is likely to play a significant role, with patients experiencing more severe poisoning potentially exhibiting slower recovery rates. Potential comorbidities, such as hepatic or renal dysfunction, can also affect the metabolism and elimination of OP compounds, thereby influencing AChE recovery. However, detailed comorbidity data was not systematically collected in this retrospective study, which is a limitation. Patient age and overall health status may also influence the body's ability to regenerate AChE.

This study has some limitations, including the small sample size and the retrospective nature of the data collection. The small sample size (n=7) limits the statistical power to detect more subtle differences in AChE recovery patterns and may affect the generalizability of the findings. Therefore, the results should be interpreted cautiously, and further research with a larger cohort is warranted. The retrospective nature of the data limited the availability of detailed, time-resolved clinical data (e.g., serial Glasgow Coma Scale scores), which hindered our ability to establish a more definitive relationship between AChE recovery and specific clinical endpoints. Clinical improvement in OP poisoning is influenced by multiple factors, including the effectiveness of supportive care, the administration of antidotes (e.g., atropine, oximes), and the patient's overall health status, in addition to AChE recovery.

Despite these limitations, our study demonstrates a general trend of AChE recovery following OP intoxication and highlights the potential differences in recovery dynamics between diazinon and malathion poisoning. Future research with a larger, prospectively collected dataset could explore the use of statistical modeling or machine learning techniques to identify predictors of AChE recovery and to develop more accurate prognostic tools.

4. Conclusions

In conclusion, this study underscores the dynamic nature of AChE activity following acute OP intoxication, demonstrating the variability in recovery patterns based on the specific OP compound. The consistent upward trend in AChE levels, particularly the robust and predictable recovery observed in diazinon cases, reinforces the clinical utility of AChE monitoring as a reliable biomarker for assessing OP intoxication progression and resolution. Our findings emphasize the

necessity of considering the specific OP compound when interpreting AChE trends and making clinical management decisions.

This research, with its focus on the nuanced differences in AChE recovery between diazinon and malathion intoxication, paves the way for future investigations aimed at identifying additional biomarkers, developing more accurate predictive models, and exploring innovative therapeutic approaches to optimize OP intoxication management. Future studies with larger sample sizes are needed to validate our observations and to establish more definitive conclusions regarding the differences in AChE recovery dynamics between diazinon and malathion poisoning.

R E F E R E N C E S

[1] *R.F. Clarke*, Insecticides: organic phosphorus compounds and carbamates in Goldfrank's Toxicologic Emergencies Eighth Ed, New York, McGraw-Hill, 2006, 1497-1512.

[2] *J. Bajgar*, Organophosphates/nerve agent poisoning: mechanism of action, diagnosis, prophylaxis, and treatment, Adv Clin Chem., Vol. **38**, 151-216, 2004, DOI: 10.1016/s0065-2423(04)38006-6.

[3] *W. Boedeker et. al*, The global distribution of acute unintentional pesticide poisoning: estimations based on a systematic review., BMC Public Health., Vol. **20**, Iss. 1, 1875, 2020.

[4] *WHO*, The global distribution of acute unintentional pesticide poisoning: estimations based on a systematic review, BMC Public Health, Vol. **20**, 1-11; DOI:10.1186/s12889-020-09939-0, 2020.

[5] *YY. Lee et al.,* The cost-effectiveness of banning highly hazardous pesticides to prevent suicides due to pesticide self-ingestion across 14 countries: an economic modelling study. Lancet Glob Health., Vol. **9,** Iss. 3, 291-300, 2021.

[6] *M. Eddleston et al.,* A role for solvents in the toxicity of agricultural organophosphorus pesticides. Toxicon, Vol. **294,** Iss. 2–3, 94-103, 2012

[7] *R. Gupta*, Handbook of Toxicology of Chemical Warfare Agents (Second Edition), Academic Press, 1071-1087, 2015, DOI: 10.1016/C2013-0-15402-5.

[8] *A. Miodovnik*, Prenatal Exposure to Industrial Chemicals and Pesticides and Effects on Neurodevelopment, in Earth Systems and Environmental Sciences, 2018, DOI:10.1016/b978-0-12-409548-9.11008-5.

[9] *J. Kaushal et. al,* A treatise on Organophosphate pesticide pollution: Current strategies and advancements in their environmental degradation and elimination, Ecotoxicology and Environmental Safety, Vol. **207**, DOI: 10.1016/j.ecoenv.2020.111483, 2021.

[10] *F. Kaloyanova and M. El Batawi*, Chapter 2 - Organophosphorous Compounds, in Human Toxicology of Pesticides, CRC Press, Taylor & Francis Group, 2019, 26-29.

[11] *P. Fina et al*, Human Toxicology of Pesticides, CRC Press, Taylor & Francis Group, 26-29, 2019.

[12] *M. Balali-Mood et. al*, Health Aspects of Organophosphorous Pesticides in Asian Countries, Iranian Journal of Public Health, Vol. **41**, Iss. 10, 1-14, 2012.

[13] *M. Balali-Mood and M. Abdollahi*, Basic and Clinical Toxicology of Organophosphorus

Compounds, Springer, 67 – 151, 2014.

[14] *F. Mohammad, A. Alias and O. Ahmed*, Electrometric measurement of plasma, erythrocyte, and whole blood cholinesterase activities in healthy human volunteers, J. Med. Toxicol., Vol. **3**, 25-30, 2007.

[15] *M. Pohanka*, Determination of acetylcholinesterase and butyrylcholinesterase activity without dilution of biological samples, Chemical Papers, Vol. **69**, Iss. 8, 1044–1049; DOI: 10.1515/chempap-2015-0117, 2015.

[16] *S. Liu and J. Pleil*, Human blood and environmental media screening method for pesticides and polychlorinated biphenyl compounds using liquid extraction and gas chromatography-mass spectrometry analysis, J. Chromatogr. B, Vol. **769**, 155-167; DOI: 10.1016/s1570-0232(01)00640-7, 2002.

[17] *S. Huber, M. Averina and J. Brox*, Automated sample preparation and GC-API-MS/MS as a powerful tool for analysis of legacy POPs in human serum and plasma, Anal. Methods, Vol. **7**, DOI: 10.1039/c9ay02400j, 2020.

[18] *J. Fang et. al*, Performance of atmospheric pressure gas chromatography-tandem mass spectrometry for the analysis of organochlorine pesticides in human serum, Anal. Bioanal. Chem., Vol. **411**, 4185–4191; DOI: 10.1007/s00216-019-01822-1, 2019.

[19] *H. Sapahin et. al*, Determination of organophosphorus pesticide residues in vegetables using solid phase micro-extraction coupled with gas chromatography–flame photometric detector, Arb. J. Chem., Vol. **12**, 1934–1944, 2019.

[20] *T. Cserhati and M. Szogyi*, Chromatographic determination of pesticides in foods and food products, J. Nutr. Food Sci., Vol. **2**, 126, DOI: 10.4172/2155- 9600.1000126, 2012.

[21] *R. Bhadekar et. al*, Development in analytical methods for detection of pesticides in environmental samples, Am. J. Anal. Chem., Vol. **2**, 1–15, DOI:10.4236/ajac.2011.228118, 2011.

[22] *I. Papoutsis et. al*, Development and validation of a simple GC-MS method for the simultaneous determination of 11 anticholinesterase pesticides in blood-clinical and forensic toxicology applications, J. Forensic Sci., Vol. **57**, 806–812, DOI:10.1111/j.1556-4029.2011.02031.x, 2012.

[23] *O. Luzardo et. al*, Validated analytical methodology for the simultaneous determination of a wide range of pesticides in human blood using GC–MS/MS and LC–ESI/MS/MS and its application in two poisoning cases, Science & Justice, Vol. **55**, Iss. 5, 307–315; DOI:10.1016/j.scijus.2015.04, 2015.

[24] *V. Honnakatti, N. Nimbal and P. Doddapattar,* A study on serum cholinesterase level in organophosphorus poisoning and its correlation with severity of organophosphorus poisoning, Int. J. Adv. Med., Vol. **5**, 1021–1025, DOI: 10.18203/2349- 3933.ijam20, 2018.

[25] *J. Fang et. al*, Performance of atmospheric pressure gas chromatography-tandem mass spectrometry for the analysis of organochlorine pesticides in human serum, Anal. Bioanal. Chem., Vol. **411**, 4185–4191, DOI: 10.1007/s00216-019-01822-1, 2019.

[26] *S. Huber, M. Averina and J. Brox*, Automated sample preparation and GC-API-MS/MS as a powerful tool for analysis of legacy POPs in human serum and plasma, Anal. Methods, Vol. **7**, DOI: 10.1039/c9ay02400j, 2020.

[27] *C. Chang et. al.*, Determination of twenty organophosphorus pesticides in blood serum by gas chromatography-tandem mass spectrometry, Analytical Methods, Vol. **8**, Iss. 22, 4487–4496, DOI:10.1039/c6ay00825a, 2016.

[28] *F. Musshoff, H. Junker and B. Madea*, Simple Determination of 22 Organophosphorous Pesticides in Human Blood Using Headspace Solid-Phase Microextraction and Gas Chromatography with Mass Spectrometric Detection, Journal of Chromatographic Science, Vol. **40**, Iss. 1, 29–39. DOI:10.1093/chromsci/40.1.29, 2002.

[29] *R. Raposo et. al*, Determination of eight selected organophosphorus insecticides in postmortem blood samples using solid-phase extraction and gas chromatography/mass spectrometry," Rapid Communications in Mass Spectrometry, Vol. **24**, Iss. 21, 3187–3194. DOI:10.1002/rcm.4765, 2010.

[30] *E. Hakme, M. Poulsen and A. Lassen,* A Comprehensive Review on Pesticide Residues in Human Urine, Journal of Agricultural and Food Chemistry, Vol. **72**, Iss. 32, 17706-17729, DOI: 10.1021/acs.jafc.4c02705. 2024.