

## REVIEW ARTICLE

# Pesticides Contamination and Analytical Methods of Determination in Environmental Matrices in Malaysia and Their Potential Human Health Effects – A Review

Siti Zulfa Zaidon<sup>1</sup>, Yu Bin Ho<sup>1</sup>, Zailina Hashim<sup>1</sup>, Nazamid Saari<sup>2</sup>, Sarva Mangala Praveena<sup>1</sup>

<sup>1</sup> Department of Environmental and Occupational Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

<sup>2</sup> Faculty of Food Science and Technology, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia

## ABSTRACT

Pesticides application is essential in protecting crops and increases their productivity. However, this causes the pesticide residues to contaminate the crops and their surrounding environment that will eventually lead to exposure to human being. There is very little understanding on the human health effects of pesticides, thus many studies are being carried out for this purpose. In conjunction to this, there are various analytical methods on multi-residue pesticides analysis that have been developed over the years in various samples. This review provides an overview of the reported concentration of pesticides, the extraction and determination techniques of pesticides in environmental samples and the potential health effects they imposed on human in Malaysia from 2007 to 2017.

**Keywords:** Multi-residue analysis, Water, Soil, Air, Sample extraction

## Corresponding Author:

Yu Bin Ho, PhD

Email: yubin@upm.edu.my

Tel: + 603 8609 2955

## INTRODUCTION

Pesticides have become a veteran key player in agriculture as it demolishes and controls pests from disturbing the production of crops. The intensive use of pesticides may not only result in high residues in the crops but also the environment via the process of leaching, run off, erosion, aerial drift or volatilization of the pesticides (1,2). In consequence, human are exposed to pesticides through various routes such as dermal, ingestion, inhalation, etc. Their ability to easily bioaccumulate in human body makes them very detrimental when exposed (3). Thus, regular monitoring of the pesticides in environmental samples is required. This is to regulate pesticide usage and limit its exposure to human and the environment. In conjunction to this, there is a necessity in finding out the pesticides usage, its health effects on human, and the viability and frequency of the extractions methods and analysis based on the types of pesticides and matrices. The aim of this review is to briefly assess the reported concentration of pesticide residues and their effects on human health, and to discuss their analytical methods in various environmental samples in Malaysia from the year

2007 to 2017. Besides that, this study also updates on the commonly detected pesticide residues in environmental matrices, sample extraction methods along with their corresponding analytical instruments, and recent studies on health effects posed by the pesticides in Malaysia for the past ten years.

## MATERIAL AND METHOD

This study compiled data on pesticide residues concentration in soil, water, personal air samples and biota, the current analytical methods used for pesticides analysis, and their effects on human health in Malaysia from 2007 to 2017. Data from various studies in Malaysia (4–21) were acquired via literature databases such as Scopus and Google Scholar. Mean concentration of pesticides compounds were reviewed along with their associated analytical methods and health effects.

## RESULTS AND DISCUSSION

A total of eleven studies were reviewed in terms of concentration (Table 1). Organochlorine pesticides (OCPs) such as dichlorodiphenyltrichloroethane family (DDTs), hexachlorocyclohexane isomers (HCHs) and endosulfan were the most frequently detected pesticides in all studies. Overall, the reported concentration were ranged from not detected (ND) to 604 µg/L in water samples, 2.55 µg/kg to 362 µg/kg in soil samples,

**Table 1:** Type of pesticides, matrix, reported concentration, type of crops, location of reviewed studies

PESTICIDES	CLASS	MATRIX	REPORTED CON-CENTRATION	CROPS	LOCATION	REFERENCE
BHCs <sup>a</sup> Chlordane Aldrin Dieldrin DDTs <sup>b</sup> Endrin Heptachlor Heptachlor epoxide Methoxychlor Endosulfan isomers Endrin aldehyde Endosulfan sulphate	Organochlorine	Water	0.015 µg/L 0.012 µg/L 0.05 µg/L 0.009 µg/L 0.63 µg/L 0.051 µg/L 0.043 µg/L ND 0.234 µg/L 0.063 µg/L ND 0.015 µg/L	n/a	Sembrong Lake, Johor	(4)
MSM <sup>c</sup> AMMT <sup>d</sup>	Sulphonylurea	Water	604.4 µg/L 55.3 µg/L	n/a	Tasik Chini, Pahang	(5)
Aldrin HCH <sup>e</sup> Heptachlor Heptachloro Epoxide Endosulfan DDTs Dieldrin Endrine Endosulfan Sulphate Diazinon Malathion Chlorpyrifos	Organochlorine        Organophosphates	Water	0.153 µg/L 0.137 µg/L 0.220 µg/L 0.513 µg/L 0.381 µg/L 0.32 µg/L 0.274 µg/L ND 0.149 µg/L 0.227 µg/L 0.421 µg/L 0.08 µg/L	organic & conventional paddy	Ledang, Johor	(6)
Hexaconazole	Azole	Soil	180 µg/kg	oil palm	Bangi, Selangor	(7)
HCHs DDTs Endosulfan	Organochlorine	Soil	5.58 µg/kg 3.78 µg/kg 8.15 µg/kg	cauliflower, cabbage and broccoli	Cameron Highlands, Pahang	(8)
HCHs DDTs	Organochlorine	Soil	8.06 µg/kg 2.55 µg/kg	flower and vegetables	Cameron Highlands, Pahang	(9)
2,4-D <sup>f</sup> Paraquat	Phenocetic acid Viologen	Soil	128 µg/kg 362 µg/kg	paddy	Kerian, Perak	(9)
HCB Lindane trans-Chlordane cis-Chlordane Dieldrin DDTs Endrin Mirex	Organochlorine	Water	0.003 µg/L 0.024 µg/L 0.003 µg/L 0.003 µg/L 0.008 µg/L 0.004 µg/L 0.002 µg/L 0.005 µg/L	n/a	Selangor river, Selangor	(11)
Quinalphos Diazinon Chlorpyrifos	Organophosphate	Water	0.03 µg/L 0.03 µg/L 0.04 µg/L	n/a	Langat river, Selangor	(12)
2,4-D Paraquat	Phenocetic acid Viologen	Water	1.49 µg/L 6.90 µg/L	paddy	Kerian, Perak	(10)
HCHs DDTs	Organochlorine	Water	0.005 µg/L 0.0009 µg/L	flowers and vegetables	Cameron Highlands, Pahang	(9)
Azoxystrobin Trifloxystrobin Buprofezin Chlorantraniliprole Difenoconazole Tebuconazole Propiconazole Tricyclazole Fipronil Imidacloprid Isoprothiolane Pretilachlor Pymetrozine	Strobin  Thiadiazin Anthranilic diamide Azole   Pyrazole Neonicotinoids Dithiolane Chloroacetan-ilide Triazine	Personal air	20.04 ng/m <sup>3</sup> 44.44 ng/m <sup>3</sup> 20.94 ng/m <sup>3</sup> 73.44 ng/m <sup>3</sup> 86.49 ng/m <sup>3</sup> 53.03 ng/m <sup>3</sup> 49.70 ng/m <sup>3</sup> 65.03 ng/m <sup>3</sup> 67.86 ng/m <sup>3</sup> 18.96 ng/m <sup>3</sup> 57.42 ng/m <sup>3</sup> 107.19 ng/m <sup>3</sup> 79.51 ng/m <sup>3</sup>	paddy	Tanjong Karang, Selangor	(13)
DDTs Endosulfan	Organochlorine	Fish, squids	22.5 µg/kg 2880 µg/kg	paddy	Tanjong Karang, Selangor	(13)

n/a: not applicable

<sup>a</sup> Hexachlorocyclohexane isomers

<sup>c</sup> Metsulfuron methyl benzoate

<sup>f</sup> 2,4-dichlorophenoxyacetic acid

<sup>b</sup> Dichlorodiphenyltrichloroethane family

<sup>d</sup> IN-A4098 (MSM metabolite)

18.96 ng/m<sup>3</sup> to 107.19 ng/m<sup>3</sup> in personal air samples and 22.5 to 2880 µg/kg in biota samples. The highest concentration (2880 µg/kg) belonged to endosulfan in marine biota samples (14), whereas both heptachlor epoxide (4) and endrine (4,6) were not detected. Eight of the compounds reported (HCHs, aldrin, dieldrin, heptachlor, chlordane, DDTs, endosulfan, quinalphos) were either banned or restricted by Pesticides Act 1974 (22), however they are widely used in various crops such as fruits like strawberries and guava, and vegetables like long beans and egg plants in Malaysia (23). It was previously reported that usage of DDTs caused mild symptoms such as nausea, dizziness, vomiting, fatigue and tremor (24). Endosulfan on the other hand had been associated with destructive effects on male reproductive system (25) and cardiovascular diseases (26). Over the course of ten years, the usage of these pesticides was not only perpetual, but increased in frequency. Regular surveillance should be done and sterner implementation should be taken by the government so that such issue will not be overseen.

### Analytical methods

Table II summarizes the analytical methods of pesticides residues in Malaysia in various matrices from the year 2007 to 2017.

### Sample preparation methods

Sample preparation is a crucial step in pesticides analysis to purify the active compound and separate it

from the matrix (27). It affects how much the residues can be salvaged from the sample matrix and contributes mostly to analysis accuracy and precision. In Table II method by Santhi and Mustafa, 2013 (11) which employed salt out and solvent extraction technique reported the lowest limit of detection (LOD) in water samples (0.0002 to 0.0028 µg/L) for fifteen OCPs without a clean-up step. Comparatively, a similar study in China (28) quantifying OCPs in water samples by also using gas chromatography coupled to electron capture detector (GC-ECD) had reported lower LOD (0.000015 µg/L). This may due to the application of solid phase extraction (SPE) as a clean-up technique that may help to improve the recovery of the OCPs. In Malaysia, the first OCPs analysis was done by Meier, Fook and Lagler, 1983 (29) on paddy fish, sediments and water. The study had applied soxhlet extraction method to extract fish and sediment samples. The method used was lengthy as soxhlet extraction takes about 4 to 6 hours to complete and required large amount of solvents; approximately 150 to 500 mL (9). Nowadays, various methods have been developed, adapted and modified to generate more conducive and efficient method (30–33). Over the years, SPE has become one of the most popular techniques for clean-up methods in various matrices such as water and foodstuffs. It requires low solvent consumption, and minimizes sample manipulation and sample load (34). The efficiency of SPE depends on the type and quantity of sorbent, the strength and volume of the elution solvent etc. (35). Although there are various sorbents available

**Table II:** Type of matrix, number of pesticides, sample extraction method, sample clean-up method, type of analytical instrument, method sensitivity of reviewed studies

MATRIX	PESTICIDE	SAMPLE EXTRACTION	SAMPLE CLEAN-UP	ANALYTICAL INSTRUMENT	SENSITIVITY	REFERENCE
Lake water River water	19 pesticides	-	SPE <sup>1</sup>	GC-ECD	-	(4)
River water Tap water	3 pesticides	-	SPE	HPLC-DAD <sup>2</sup>	LOD 0.003-0.006 µg/L	(12)
Paddy water	17 pesticides	-	SPE	GC-ECD <sup>3</sup>	LOD 0.01 -0.088 µg/L LOQ 0.035-0.263 µg/L	(6)
Lake water	2 pesticides	-	SPE	LC-VWD <sup>4</sup>	-	(5)
River water	2 pesticides	-	SPE	GC-µECD <sup>5</sup>	-	(15)
River water	15 pesticides	ethyl acetate/dichloromethane mixture (1:1)	-	GC-MS <sup>6</sup>	LOD 0.0002-0.0028 µg/L	(11)
Soil	8 pesticides	Soxhlet extraction with NaOAc <sup>7</sup> & 1:1 Acetone: Hexane	SPE	GC-ECD	LOD 0.04– 5 µg/kg	(8)
Soil (oil palm plantation)	1 pesticide	DCM <sup>8</sup> followed by filtration through sodium sulfate on filter paper	-	GC-ECD	LOD 0.2 µg/kg LOQ 1.0 µg/kg	(7)
Poultry manures	3 pesticides	SDIE <sup>9</sup>	SPE	UPLC-PDA <sup>10</sup>	-	(16)
Soil	1 pesticide	water: acetonitrile, 1:2	-	GC-ECD	-	(17)
Air	13 pesticides	acetone	-	UPLC-MS/MS <sup>11</sup>	LOD 0.1–1.0 ng/m <sup>3</sup> LOQ 0.5–2.5 ng/m <sup>3</sup>	(13)
Fish, squids	2 pesticides	LLE <sup>12</sup>	SPE	GC-MS	LOQ 0.50 to 10.00 µg/kg	(15)

<sup>1</sup> Solid phase extraction

<sup>2</sup> High-performance liquid chromatography coupled to diode array detector

<sup>4</sup> Liquid chromatography coupled to variable wavelength detector

<sup>6</sup> Gas chromatography coupled to mass spectrometry

<sup>7</sup> Sodium acetate

<sup>8</sup> Dichloromethane

<sup>3</sup> Gas chromatography coupled to electron capture detector

<sup>5</sup> Gas chromatography coupled to microelectron capture detector

<sup>9</sup> Solvent direct-immersion extraction

<sup>11</sup> Ultra-high performance liquid chromatography coupled to tandem mass spectrometry

<sup>10</sup> Ultra-performance liquid chromatography coupled to photodiode array

<sup>12</sup> Liquid-liquid extraction

commercially, developing an effective method may be difficult for both extraction and separation steps (11) in more complex matrix like soil. The low volume of solvents used may result in insufficient elimination of interferences which may cause low recovery. Another frequently-used clean-up technique that is adapted from SPE is dispersive solid phase extraction (DSPE) which employs the same technique but with different execution. The procedure includes addition of sorbent substance into the extract and further partitioned the target analytes from the matrix by centrifugation (36). DSPE is also commonly used as part of the clean-up technique for Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method. It was designed to be fast, simple, economical and viable to various type of solid samples and analytes (37–39).

In Table II, five studies on water and two studies on soil, and one study on marine biota had applied SPE for sample clean-up while the rest adapted direct injection into the analytical instrument. Other countries has widely afforded the QuEChERS technique coupled to DSPE in multi-residue analysis of pesticides in foodstuffs (40–42) and recently soil samples (38,43,44). However, none of the studies reviewed in Malaysia employed the QuEChERS-DSPE method in soil samples. In Thailand, Arnnok et al. 2017 (37) has developed polyaniline-modified zeolite NaY DSPE sorbent for multi-class pesticides extraction in food and environmental samples. The sorbent showed better recovery compared to C18 sorbent.

#### *Analytical instruments*

Gas chromatography (GC) and liquid chromatography (LC) coupled with selective detectors such as mass spectrometry (MS), diode array detector (DAD) and electron capture detector (ECD) have been used extensively in pesticides analysis. GC-ECD was first introduced in 1960s and since then was further improve to be more thermostable making it highly sensitive for analysis of halogenated compounds. It acts as a pioneer that induce the development of other detectors (45). Table II suggests that GC-ECD is the most popular instrument for pesticides analysis especially in soil and water samples. This may be due to its ability to quantify and detect non-polar, volatile ad halogenated compounds especially OCs (46). LC coupled to mass spectrometry (MS) detector associates with high sensitivity and selectivity without implying derivatization step. Globally, numerous studies had successfully employed liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) to analyse wide range of pesticides in soil and water samples (38,43,47–49). However, in Table II none of the studies on soil and water samples utilize this instrument even though recent studies have proven that this technique provides suitable selectivity for the analysis of organic contaminants in complex matrices (50). LC-MS/MS is comparatively more complex, labour-intensive and

expensive to maintain. Besides that, it often requires a concentration step to improve sensitivity of detection, this process may contribute to loss of target compounds (46).

#### *Method sensitivity*

Limit of detection (LOD) and limit of quantification (LOQ) in various environmental matrices in Malaysia are summarised in Table II. The lowest LOD in water samples was 0.0002 µg/L using GC-MS (11). Whereas, the highest LOD in water was 0.088 µg/L using GC-ECD (6). The two studies in soil samples (7,8) also utilized GC-ECD for analysis, however, method proposed by Farina et al, (2016) (8) shows lower LOD (0.04 µg/kg to 5 µg/kg) compared to Maznah et al, (2015) (0.2 µg/kg) (7). A similar study in China was reported by Liu et al. (2016) (51) which employed the same clean up technique and instrument. Liu et al, (2016) shows slightly higher LOD (1 µg/kg to 5 µg/kg) as compared to Farina et al, (2016).

#### **EFFECTS ON HUMAN HEALTH**

There were not many data on pesticides effects on human health available in the last ten years for this review, having said that, four studies were identified from 2007 to 2017 (18–21) suggested the effects the pesticides imposed on human health. Based on Table III exposure of pesticides increased the risk of cardiovascular (CVS) diseases, genotoxic, and reduced semen quality. All four studies utilized biological markers to associate the pesticides exposure to its human health effects. Samsuddin et al, 2016 (18) reported that the workers who are chronically exposed to low dose mix-pesticides are possibly at higher risk of cardiovascular diseases. A study by Xu et al. 2017 (26) also reported the association of endosulfan and CVS. The study successfully proven that endosulfan causes overexpression of a protein from the matrix metalloproteinase family (MMP) called stromelysins (MMP-3). Overexpression of MMP-3 in turn, may degenerate proteins such as fibronectin, laminin, and cartilage proteoglycans. These proteins are involved in progression of atherosclerosis (52). Sutris et al, 2016 (19) had conducted a genotoxicity study of organophosphates on Orang Asli children living in an agricultural island in Kuala Langat, Selangor. The study reported that the organophosphate genotoxicity among children is associated with the organophosphates detected in urine and the residential period in the agricultural village. Children with detectable urinary organophosphates had a longer comet tail length compared to children with undetectable urinary organophosphates. Those who had lived in the agricultural island for ten years and more had significantly longer comet tail length. Comets are the resulting images of the comet assay that is conducted in genotoxicity studies. Increase in comet tail length indicates the extent of the DNA damage (50). Similarly, studies in India (53) and Croatia (54) suggested a positive association between pesticides exposure level and comet tail length. Hossain et al, 2010 (21) had reported significant association between pesticides exposure and

**Table III:** Exposed population, health effects, type of biological markers of reviewed studies

EXPOSED POPULATION	PESTICIDES	HEALTH EFFECTS	BIOLOGICAL MARKER	REFERENCE
Workers	Mixture of pesticides: fenitrothion , malathion, primiphos methyl, permethrine, cyfluthrin, deltamethrin	Chronic mix-pesticide exposure among workers is associated with CVS hemodynamic parameters	Diazoxonase and the increase in ox-LDL, brachial and aortic DBP and SBP, and heart rate.	(18)
Children	Organophosphates	Amount of exposure and length of residence is associated with organophosphate genotoxicity	Organophosphate pesticides urinary metabolites	(19)
Farmers	n/a	Pesticide and fertilizers exposure may contribute to the promotion of nuclear anomalies	Micronucleus (MN) and binucleus (BNu) frequencies	(20)
Farmers	Paraquat, malathion	A significant decline in semen quality among subjects with pesticide exposure	Volume, pH, sperm concentration, motility, morphology and WBC count	(21)

semen quality. This finding is supported by a number of comparable studies that had reported in low sperm count (55,56), changes in sperm motility, morphology and concentration (57) due to pesticides exposure. An animal study in Kuwait in 2006, found that exposure of rats to organophosphate, methyl parathion (MP) had resulted in disruption in testosterone and Luteinizing Hormone (LH) in the testis, and had induced tubular atrophy by causing cell death and the formation of multinucleated cells in the testis (58).

## CONCLUSION

Although pesticides have shown great importance in providing good quality and protection to the crops, their disadvantages pose the same degree of concern in human and environment as it affects the natural resources and natural populations. Usage of restricted pesticides have been widely applied by local farmers indicating that the pesticides can be easily obtained or purchased regardless of the regulation outlined by the authority. Thus, more enhanced enforcement should be conducted in order to manage and prevent the extensive use of these pesticides.

Monitoring the trace levels of pesticides in environmental samples is an essential step of enforcement, however developing a reliable and efficient method for multiple pesticides analysis with high sensitivity and accuracy may be difficult as many aspects should be considered. Numerous techniques have been introduced and improved over the years to minimize the loss of analytes, time, cost and the tedium of a sample preparation method. Besides that, more sensitive analytical instruments have also been introduced in this line of study in order to aid the achievement of an optimum method. Advanced analytical instruments such as LC-MS/MS and GC-MS/MS should be more employed in environmental samples in Malaysia in order to improve LOD and LOQ of existing methods. From 2007 to 2017,

there are not many studies on method development multi-residue analysis of pesticides in Malaysia. Existing methods show acceptable sensitivity when applied to environmental samples.

## ACKNOWLEDGEMENTS

Financial assistance that was provided by Putra Grant (GP-IPS/2016/9478100) to carry out this work is thankfully acknowledged. The authors are grateful to all studies reviewed in this paper for the information provided in their studies.

## REFERENCES

1. Akdogan A, Hol A, Divrikli U, Elci L, Campus I. Determination of pesticides in soil by mechanical stirring-assisted extraction coupled with gas chromatography-mass spectrometry. *Anal Lett.* 2014;47(4):675–88.
2. Gavrilescu M. Fate of pesticides in the environment and its bioremediation. *Eng Life Sci [Internet].* 2005 Dec [cited 2014 Oct 25];5(6):497–526. Available from: <http://doi.wiley.com/10.1002/elsc.200520098>
3. Fenik J, Tankiewicz M, Biziuk M. Properties and determination of pesticides in fruits and vegetables. *TrAC Trends Anal Chem [Internet].* Elsevier Ltd; 2011 Jun [cited 2014 Nov 4];30(6):814–26. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0165993611000938>
4. Sharip Z, Hashim N, Suratman S. Occurrence of organochlorine pesticides in a tropical lake basin. *Environ Monit Assess. Environmental Monitoring and Assessment;* 2017;189:560(11).
5. Jansar K., Sahid I. RESIDUE DETERMINATION AND MONITORING OF THE LEVELS OF METSULFURON METHYL IN SELECTED RIVERS AT TASIK CHINI, Pahang, Malaysia. *Malaysian J*

- Anal Sci. 2016;20(1):179–86.
6. Abdullah P, Rozali M, Ishak A, Jaber K. Detection of Pesticides Residues in Water Samples from Organic and Conventional Paddy Fields of Ledang, Johor, Malaysia. In: THE 2016 UKM FST POSTGRADUATE COLLOQUIUM. 2016.
  7. Maznah Z, Halimah M, Ismail S. Dissipation of the fungicide hexaconazole in oil palm plantation. *Environ Sci Pollut Res*. 2015;22:19648–57.
  8. Farina Y, Abdullah M., Bibi N, Khalik WMAW. Pesticides residues in agricultural soils and its health assessment for humans in cameron highlands, Malaysia. *Malaysian J Anal Sci*. 2016;20(6):1346–58.
  9. Saadati N, Abdullah P, Zakaria Z, Rezayi M, Hosseinizare N. Distribution and fate of HCH isomers and DDT metabolites in a tropical environment – case study Cameron Highlands-Malaysia. *Chem Cent J*. 2012;6.
  10. Ismail BS, Sameni M, Halimah M. Evaluation of herbicide pollution in the kerian ricefields of Perak, Malaysia. *World Appl Sci*. 2011;15(1):5–13.
  11. Santhi VA, Mustafa AM. Assessment of organochlorine pesticides and plasticisers in the Selangor River basin and possible pollution sources. *Environ Monit Assess*. 2013;185:1541–54.
  12. Wee SY, Omar TFT, Aris AZ, Lee Y. Surface Water Organophosphorus Pesticides Concentration and Distribution in the Langat River, Selangor, Malaysia. *Expo Heal* [Internet]. Springer Netherlands; 2016;8(4):497–511. Available from: "http://dx.doi.org/10.1007/s12403-016-0214-x
  13. Hamsan H, Bin Y, Zulfa S, Hashim Z, Saari N, Karami A. Occurrence of commonly used pesticides in personal air samples and their associated health risk among paddy farmers. *Sci Total Environ* [Internet]. Elsevier B.V.; 2017;603-604:381–9. Available from: <https://doi.org/10.1016/j.scitotenv.2017.06.096>
  14. Santhi VA, Hairin T, Mustafa AM. Simultaneous determination of organochlorine pesticides and bisphenol A in edible marine biota by GC – MS. *Chemosphere* [Internet]. Elsevier Ltd; 2012;86(10):1066–71. Available from: <http://dx.doi.org/10.1016/j.chemosphere.2011.11.063>
  15. Haron S., Ismail BS. Comparison pesticide residue levels in the surface of Bertam River in Cameron Highlands, Pahang. In: AIP Proceedings. 2015.
  16. Ong S, Hafiz A, Majid A, Ahmad H. Insecticide Residues on Poultry Manures: Field Efficacy Test on Selected Insecticides in Managing *Musca Domestica* Population. *Trop Life Sci Res*. 2017;28(2):45–55.
  17. Ismail BS, Mazlinda M, Tayeb M. The Persistence of Deltamethrin in Malaysian Agricultural Soils. *Malaysiana, Sains*. 2015;44(3):83–9.
  18. Samsuddin N, Rampal KG, Ismail NH, Abdullah NZ, Nasreen HE. Pesticide Exposure and Cardiovascular Hemodynamic Parameters Among Male Workers Involved in Mosquito Control in East Coast of Malaysia. *Am J Hypertens*. 2016;29(February 2016):226–33.
  19. Sutris JM, How V, Sumeri SA, Mohammad H, Ghazi HF, Isa ZM. Genotoxicity following Organophosphate Pesticides Exposure among Orang Asli Children Living in an Agricultural Island in Kuala Langat, Selangor, Malaysia. *Int J Occup Environ Med*. 2016;7:42–51.
  20. Hamid ZA, Faizal M, Zulkifly M, Hamid A, Lubis SH, Mohammad N, et al. The association of nuclear abnormalities in exfoliated buccal epithelial cells with the health status of different agricultural activities farmers in Peninsular Malaysia. *Genes Environ* [Internet]. Genes and Environment; 2016;38:1–9. Available from: <http://dx.doi.org/10.1186/s41021-016-0032-1>
  21. Hossain F, Li OA, Ouza UJADS, Khin D, Aing SN. Effects of Pesticide Use on Semen Quality among Farmers in Rural Areas of Sabah, Malaysia. *J Occup Heal*. 2010;52:353–60.
  22. PESTICIDES ACT 1974. Laws of Malaysia; 2015.
  23. Zaidon SZ, Hamsan H, Bin HY. A Review on Pesticides Occurrence in Fruits and Vegetables in Malaysia and Their Potential Health Risk Among Adults. *Indian J Environ Prot*. 2016;36(January):826–32.
  24. Casarett LJ, Klaassen CD, Amdur MO, Doull J. Casarett and Doull's toxicology: the basic science of poisons. In: Klaassen CD, editor. Fifth edit. New York: McGraw-Hill, Health Professions Division; 1996. p. 20894.
  25. Saiyed H, Dewan A, Bhatnagar V, Shenoy U, Shenoy R, Rajmohan H. Children's Health I Article Effect of Endosulfan on Male Reproductive Development. *Environ Health Perspect*. 2003;111(16):1958–62.
  26. Xu D, Liu T, Lin L, Li S, Hang X, Y S. Exposure to endosulfan increases endothelial permeability by transcellular and paracellular pathways in relation to cardiovascular diseases. *Environ Pollut*. 2017;223:111–9.
  27. Masić A, Vésquez K, Campo J, Picy Y. Assessment of two extraction methods to determine pesticides in soils, sediments and sludges. Application to the Tŕria River Basin. *J Chromatogr A* [Internet]. Elsevier B.V.; 2014;1378:19–31. Available from: <http://dx.doi.org/10.1016/j.chroma.2014.11.079>
  28. Xue N, Xu X, Jin Z. Screening 31 endocrine-disrupting pesticides in water and surface sediment samples from Beijing Guanting reservoir. *Chemosphere*. 2005;61:1594–606.
  29. Meier PG, Fook DC, Lagler KF. Organochlorine Pesticide Residues in Rice Paddies in Malaysia, 1981. *Bull Environ Contam Toxicol*. 1983;30:351–7.
  30. Kadir HA, Abas F, Zakaria O. Analysis of pesticide residues in tea using accelerated solvent extraction with in-cell cleanup and gas chromatography

- tandem mass spectrometry. *Anal Methods*. 2015;7:3141–7.
31. Vijaya BRA, Yusop Z, Jaafar J, Aris A Bin, Majid ZA, Umar K. Simultaneous determination of three organophosphorus pesticides in different food commodities by gas chromatography with mass spectrometry. *J Separation Sci*. 2016;39(12):2276–83.
  32. Zainudin BH, Salleh S, Mohamed R, Yap KC, Muhamad H. Development , validation and determination of multiclass pesticide residues in cocoa beans using gas chromatography and liquid chromatography tandem mass spectrometry. *Food Chem [Internet]*. Elsevier Ltd; 2015;172:585–95. Available from: <http://dx.doi.org/10.1016/j.foodchem.2014.09.123>
  33. Zainudin BH, Salleh S. Method Development , Optimization and Validation of Matrix Hydration Effect on Pesticide Residues in Cocoa Beans Using Modified QuEChERS Method and Gas Chromatography Tandem Mass Spectrometry. *Food Anal Methods*. *Food Analytical Methods*; 2017;10:3874–85.
  34. Picy Y, Fernández M, Ruiz MJ, Font G. Current trends in solid-phase-based extraction techniques for the determination of pesticides in food and environment. *J Biochem Biophys Methods*. 2007;70:117–31.
  35. Yang X, Zhang H, Liu Y, Wang J, Zhang YC, Dong a J, et al. Multiresidue method for determination of 88 pesticides in berry fruits using solid-phase extraction and gas chromatography-mass spectrometry: Determination of 88 pesticides in berries using SPE and GC-MS. *Food Chem [Internet]*. Elsevier Ltd; 2011 Jul 15 [cited 2014 Nov 10];127(2):855–65. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23140746>
  36. Fontana AR, Camargo A, Martinez LD, Altamirano JC. Dispersive solid-phase extraction as a simplified clean-up technique for biological sample extracts . Determination of polybrominated diphenyl ethers by gas chromatography – tandem mass spectrometry. *J Chromatogr A [Internet]*. Elsevier B.V.; 2011;1218(18):2490–6. Available from: <http://dx.doi.org/10.1016/j.chroma.2011.02.058>
  37. Annok P, Patdhanagul N, Burakham R. Dispersive solid-phase extraction using polyaniline-modified zeolite NaY as a new sorbent for multiresidue analysis of pesticides in food and environmental samples. *Talanta [Internet]*. Elsevier; 2017;164(August 2016):651–61. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0039914016308657>
  38. Caldas SS, Bolzan M, Cerqueira MB, Furlong EB, Fagundes C, Primel EG. Evaluation of a Modified QuEChERS Extraction of Multiple Classes of Pesticides from a Rice Paddy Soil by LC-APCI-MS/MS. *J Agric Food Chem*. 2011;59:11918–26.
  39. Li R, Wu M. High-Throughput Determination of 30 Veterinary Drug Residues in Milk Powder by Dispersive Solid-Phase Extraction Coupled with Ultra-High Performance Liquid Chromatography Tandem Mass Spectrometry. *Food Anal Methods*. *Food Analytical Methods*; 2017;10:3753–62.
  40. Carneiro RP, Oliveira FAS, Madureira FD, Silva G, Souza WR De, Pereira R. Development and method validation for determination of 128 pesticides in bananas by modified QuEChERS and UHPLC e MS/MS analysis. *Food Control*. 2013;33:413–23.
  41. Correia-s L, Domingues VF, Delerue-matos C. Validation of QuEChERS method for organochlorine pesticides analysis in tamarind (*Tamarindus indica*) products : Peel , fruit and commercial pulp. *Food Control*. 2015;54:374–82.
  42. Wang J, Chow W, Leung D. Applications of LC/ESI-MS/MS and UHPLC QqTOF MS for the determination of 148 pesticides in fruits and vegetables. *Anal Bioanal Chem [Internet]*. 2010 Feb [cited 2014 Nov 27];396(4):1513–38. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20063156>
  43. Abdel-ghany MF, Hussein LA, El NF, El-khatib AH, Linscheid MW. Simultaneous determination of eight neonicotinoid insecticide residues and two primary metabolites in cucumbers and soil by liquid chromatography – tandem mass spectrometry coupled with QuEChERS. *J Chromatogr B [Internet]*. Elsevier B.V.; 2016;1031:15–28. Available from: <http://dx.doi.org/10.1016/j.jchromb.2016.06.020>
  44. Martins GL, Friggi C a., Prestes OD, Vicari MC, Friggi D a., Adaime MB, et al. Simultaneous LC-MS/MS Determination of Imidazolinone Herbicides Together with Other Multiclass Pesticide Residues in Soil. *CLEAN - Soil, Air, Water [Internet]*. 2014 Oct 12 [cited 2015 Jan 7];42(10):1441–9. Available from: <http://doi.wiley.com/10.1002/clen.201300140>
  45. Hoff V Der, Zoonen P Van, Rene G. Trace analysis of pesticides by gas chromatography. *J Chromatogr A*. 1999;843:301–22.
  46. Samsidar A, Shaarani S. Trends in Food Science & Technology A review of extraction , analytical and advanced methods for determination of pesticides in environment and foodstuffs. 2018;71(November 2017):188–201.
  47. Gerynimo E De, Aparicio VC, B6rbaro S, Portocarrero R, Jaime S, Costa JL. Presence of pesticides in surface water from four sub-basins in Argentina. *Chemosphere*. 2014;107:423–31.
  48. Demoliner A, Caldas SS, Costa FP, Gonzalves FF. Development and Validation of a Method using SPE and LC-ESI-MS-MS for the Determination of Multiple Classes of Pesticides and Metabolites in Water Samples. *J Braz Chem Soc*. 2010;21(8):1424–33.
  49. Li Y, Qiao L, Li F, Ding Y, Yang Z, Wang M. Determination of multiple pesticides in fruits and vegetables using a modified quick , easy , cheap , effective , rugged and safe method with magnetic

- nanoparticles and gas chromatography tandem mass spectrometry. *J Chromatogr A* [Internet]. Elsevier B.V.; 2014;1361:77–87. Available from: <http://dx.doi.org/10.1016/j.chroma.2014.08.011>
50. Montagner CC, Vidal C, Acayaba RD, Jardim WF, Jardim ICSF, Umbuzeiro G a. Trace analysis of pesticides and an assessment of their occurrence in surface and drinking waters from the State of Sro Paulo (Brazil). *Anal Methods* [Internet]. Royal Society of Chemistry; 2014 Jun 30 [cited 2014 Nov 9];6(17):6668. Available from: <http://xlink.rsc.org/?DOI=C4AY00782D>
  51. Liu Y, Li S, Ni Z, Qu M, Zhong D, Ye C, et al. Pesticides in persimmons, jujubes and soil from China: Residue levels, risk assessment and relationship between fruits and soils. *Sci Total Environ* [Internet]. Elsevier B.V.; 2016;542:620–8. Available from: <http://dx.doi.org/10.1016/j.scitotenv.2015.10.148>
  52. Kunz J. Matrix Metalloproteinases and Atherogenesis in Dependence of Age. *Gerontology*. 2007;53:63–73.
  53. Yadav AS, Sehrawat G. Evaluation of Genetic Damage in Farmers Exposed to Pesticide Mixtures. *J Hum Genet*. 2011;11(2):105–9.
  54. Garaj-vrhovac V, Zeljezic D. Evaluation of DNA damage in workers occupationally exposed to pesticides using single-cell gel electrophoresis ( SCGE ) assay Pesticide genotoxicity revealed by comet assay. *Mutat Res*. 2000;469:279–85.
  55. Tuc VP, Wangsuphachart V, Tasanapradit P, Fungladda W. IMPACTS OF PESTICIDE USE ON SEMEN CHARACTERISTICS AMONG RICE FAMERS IN KIENXUONG DISTRICT , THAIBINH. *Southeast Asian J Trop Med Public Heal*. 2007;38(3):569–75.
  56. Lerda D, Rizzi R. Study of reproductive function in persons occupationally exposed to 2 , 4-dichlorophenoxyacetic acid ( 2 , 4-D ). *Mutat Res*. 1991;262:47–50.
  57. Dalvie MA, Myers FJE, Thompson L, Robins TG, Dyer S, Riebow J, et al. The long-term effects of DDT exposure on semen , fertility , and sexual function of malaria vector-control workers in Limpopo Province , South Africa. *Environ Res*. 2004;96:1–8.
  58. Narayana K, Prashanthi N, Nyanatara A, Bairy LK, Souza UD. An organophosphate insecticide methyl phosphorothioate ) induc .... *J Toxicol Sci*. 2006;31(3):177–89.