

Toxicological analysis of acute pesticides poisoning among Moroccan population

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Abstract. The aim of this study is to evaluate the importance of toxicological analysis in acute intoxications by pesticides through the experience of the laboratory of the Poison Control and Pharmacovigilance Center of Morocco (CAPM). This is a retrospective study from January 2014 to September 2020, concerning suspected pesticides poisoning cases. The cholinesterase activity was measured in whole blood and the chromatographic analyzes (GC-MS and LC-MS-MS) were conducted in blood, urine and gastric lavage fluid. In this study, 398 tests were involved. The median age of the patients was 14 with an interquartile range of [4-26 years]. The sex ratio (F / M) was 1.5. The requests were from the region of Rabat-Salé-Kénitra in 82.8% of cases (north-western Morocco). The cholinesterase activity measurement was performed for 295 patients with low values in 3.7% of cases. The toxicological screening by GC-MS and LC-MS-MS detected the pesticide responsible of poisoning in 62 cases as insecticides in 38% of cases, redenticides (33% of cases) and herbicides (5% of cases). Toxicological analysis plays an essential role in the diagnosis and the management of acute pesticides poisoning. Interpretation of results is carried over by analyst with close cooperation with clinical toxicologist.

Introduction

Pesticides are used across the world to protect crops, plants in agriculture, gardens or domestics, against insects, rodents or any pests threatening the public health. Indeed, this chemical molecules causes above of 5 million of intoxications annually, according to the World Health Organization (WHO) [1]. Toxicity effects depend on the dose of the substance consumed and the health stat of the poisoned person. The effects are reduced with time when the exposition is limited or removed. The recovery is possible if no health complication has been occurred [2]. The misuse of the pesticides is the main cause of this type of intoxication. Therefore, the suicide is the main cause worldwide [1]. In Morocco, the pesticides intoxications are in the front line among all intoxications according to the Poison Control and Pharmacovigilance Centre of Morocco (CAPM). The same center reported, 11196 acute pesticides poisoning cases, in Morocco from 2008 to 2016 [3].

Nowadays, analytical methods, especially chromatographic ones, facilitate the detection and the quantification of pesticides in biological samples and contribute in the diagnostic and the management of acute poisoning cases. Therefore, the aim of this study is to

highlight the role of the toxicological analysis in the management of acute intoxication by pesticides among Moroccan population.

1 Method

This is a retrospective study conducted in Morocco describing 398 cases of acute poisoning with pesticides reported to the laboratory of the CAPM between January 2014 and September 2020. The data collected was from the laboratory registration system of analysis requests received from Moroccan hospitals. We were interested to describe the requests using the demographic characteristics (age, sex, date of analysis, provenance) and the toxicological characteristics (symptomatology and pesticide name). Classification of pesticides was performed based on the chemical composition.

After the reception, the sample is labeled and directed to extraction before the chromatographic analysis. For the blood, a cholinesterasic measurement is operated before and centrifuged to acquire the plasma for the next step. In the study period, this measurement used two technics; Test-mate ChE Cholinesterase Test System (Model 400) and ChE check mobile. After a liquid-liquid extraction, a first large screening by Liquid Chromatography with

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tandem mass spectrometry (LC-MS-MS) and Gas Chromatography with mass spectrometry (GC-MS), then a confirmation step with the specific mass ion.

1.1 Apparatus

1.1.1 GC-MS

The analysis are executed with the 680 PerkinElmer Clarus GC-MS and a column RESTEK RXI® 5ms (30 m × 0.25 mm, 250 µm). The carrier gas is helium with a flow rate: 0.1 ml/min.

Mass spectrometry detector used an electron ionization mode with an ionization energy of 70 eV. The ion source temperature was 250 °C.

1.1.2 LC-MS-MS

3200Q TRAP-AB Sciex Instrument was used with analysis time of 15 minutes and injection volume of 10 µl. The aqueous phase is composed by water, ammonium formate (1M) and formic acid (0.1M). For the organic phase, we replaced the water by the methanol.

1.1.3 Solvents

The chromatographic analysis used methanol (99.9%) and the ammonium format (99.99%) purchased from Sigma Aldrich®. We purchased Formic acid (99%) and dichloromethane (99.9%) from Carlo Ebra, propanol-2 (99.9%) from Panreac and heptane (99%) from Scharlar.

1.1.4 Extraction

We added one milliliter of the sodium carbonate buffer to 1 ml of the sample (plasma, urine or gastric liquid). Then, 5 ml of the extraction solvent (Propanol, Dichloromethane and formic acid) was added to the preparation after a quick agitation [4]. The whole is centrifuged for 10 min. The eluent is evaporated under nitrogen gas at 45 °C. The recovery from the evaporation tubes is done with methanol. Only 10 µl are injected into the GC-MS and the LC-MS-MS.

1.1.4 Data analysis

The data analysis of GC-MS was done with TurboMass® software version 6.0 and the NIST library version 2008. The LC-MS-MS results were given by Analyst®1.6.2 software (version 2013), using Multi Reaction Monitoring (MRM) of each molecule, retention time (t_R) and the concordance in the library.

2 Results

2.1 Demographic characteristics of the study population

398 cases of suspected pesticides poisoning from Moroccan hospitals were studied. The median age of the patients was 14 years with an interquartile range of [4-26 years]. The poisoning cases concerned children in accidental circumstance in 51% of all cases (Figure 1).

We found that 60% of the patients are female (Figure 2) with a sex ratio (F/M) equal to 1.5. From 2014 to September 2020, the number of cases varied from 47 to 73 per year (Figure 3). Because of the proximity, almost all the cases (82.8%) was from north-western of Morocco (Rabat-Salé-Kénitra).

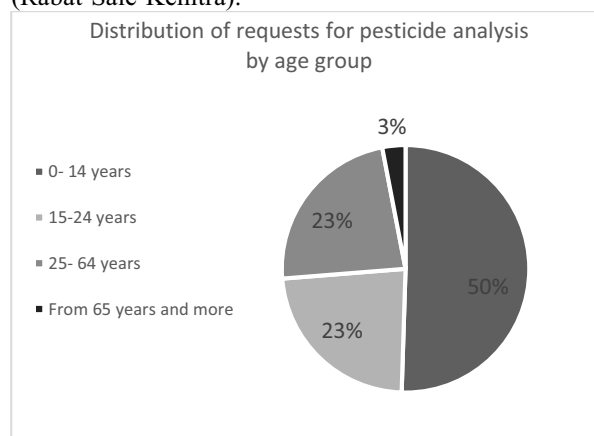


Fig. 1. Distribution of requests for pesticide analysis by age group.

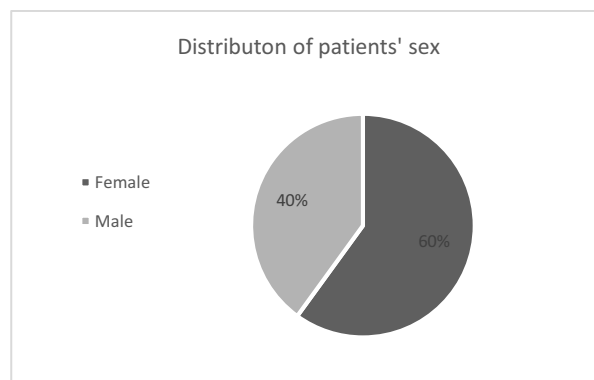


Fig. 2. Distribution of patients' sex

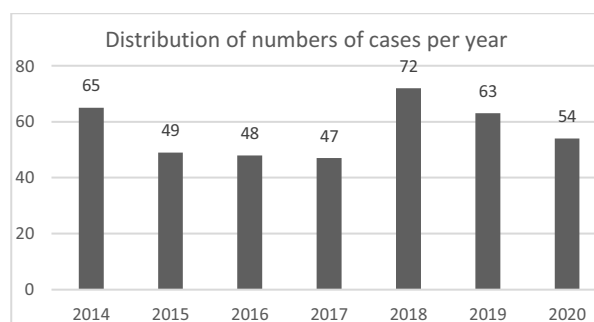


Fig. 3. Distribution of numbers of cases per year

2.2 Cholinesterase measurement

This measurement is systematically done for any suspicion of poisoning by organophosphates (Op) or carbamates (Cbt). We did 295 measurement with two photometric technics, Method 1 (M 1) and Method 2 (M2), with the same principle. Statistical analysis of the results is done (Table 1).

For the Test System (Model 400) technic, the results are given as percentages. A severe intoxication indicates values between 25% and 0%. It may be important to consider values from 25% to 50% because it directs to a probable severe poisoning, repetition of the measurement is recommended in this situation.

The ChE check mobile, an interval of normal values is followed. For AChE the results must be between 33.0 and 49.4 U/gHb. The BChE is normal if its values are up to 1623. and under 3861 U/L.

A correlation between age, AChE and BChE was done. We illustrate the correlation for the two methods in the figures (Figures 4 to 9). The strongest correlation is verified with a coefficient correlation close to 1 or -1. When the variables are not correlated, the coefficient is near to zero. For our results, the coefficients of correlation are respectively; 0.0002, 0.0167, 0.3196, 0.0004, 0.0722 and 0.2393. Then, no correlation is observed.

The laboratory received blood samples more than once for outcome cholinesterase monitoring.

Table 1. Statistical analysis of AChE and BChE measured in blood by two methods

Parameters	Statistics	F	M
Age (years)	N	129	85
	Max	76	78
	Min	0	0.2
	Average	18	18
AChE1 (%)	Max	94	100
	Min	<10	<5
	Average	48.7	47.4
	Standard deviation	23.3	24.3
BChE1 (%)	Max	100	106
	Min	<10	-14
	Average	60.71	54.03
	Standard deviation	28.65	28.5
AChE2	Max	53.5	82.6
	Min	4.44	6.2
	Average	40.85	43.06
	Standard deviation	17.7	15.23
BChE2	Max	5100	5939.8
	Min	377	361.9
	Average	2310.96	2453.6

	Standard deviation	2285.69	2293.19
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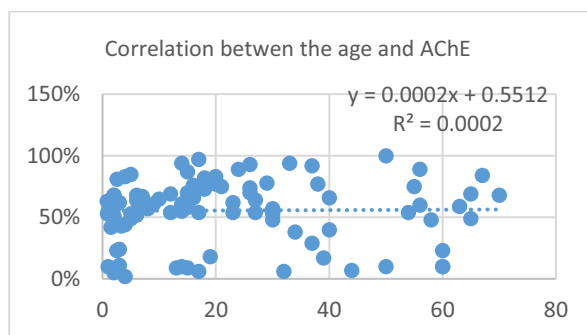


Fig. 4: Correlation between the age and AChE (M 1)

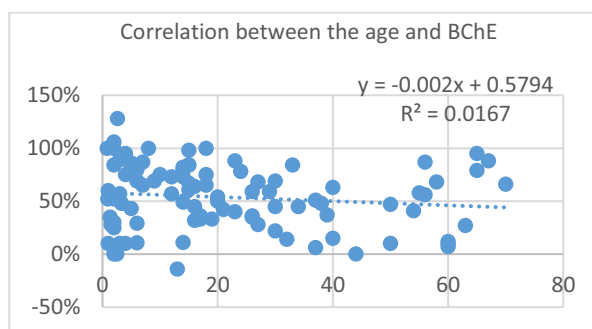


Fig. 5: Correlation between the age and BChE (M1)

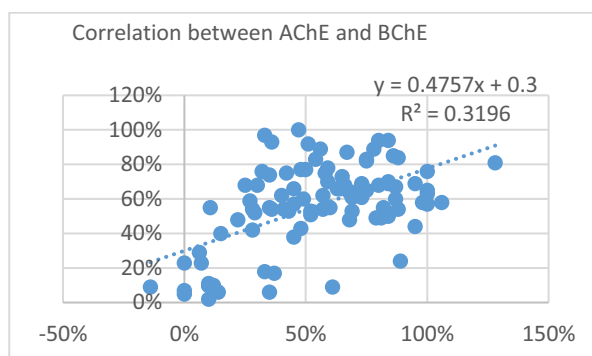


Fig. 6: Correlation between AChE and BChE (M 1)

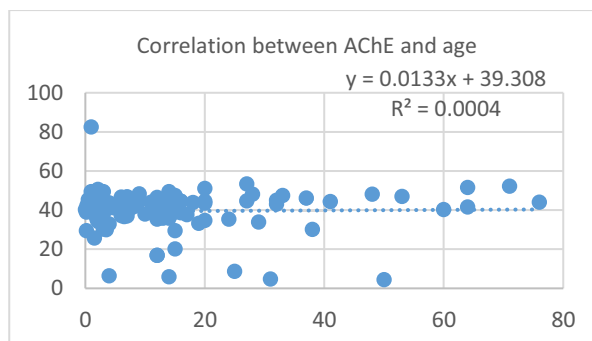


Fig. 7: Correlation between the age and AChE (M 2)

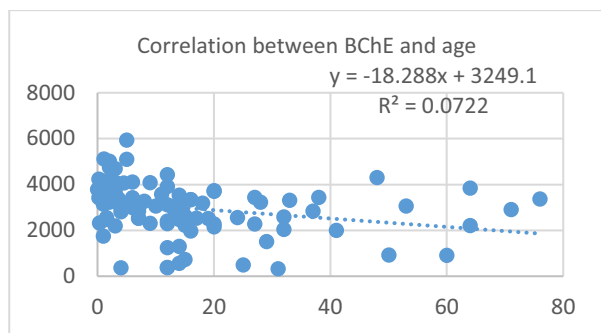


Fig. 8: Correlation between the age and BChE (M 2)

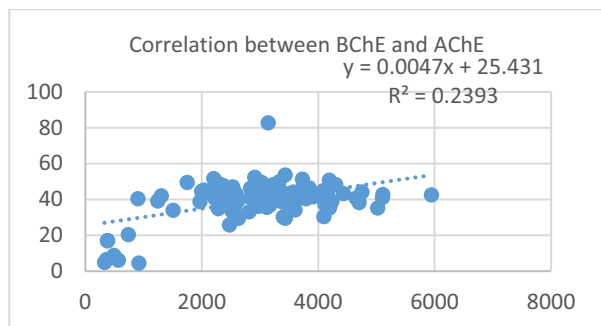


Fig. 9: Correlation between AChE and BChE (M 2)

2.3 Chromatographic analysis

It is 360 chromatographic analysis. It confirmed the presence of pesticides among 62 poisoned patients. The results shows families of pesticides responsible of poisoning in 62 cases (Figure 10). The insecticides were the first family (38%), with Op in (58%), Cbt (33%) and pyrethroids (24%). The rodenticides (33%) were in the second range, all the patients were poisoned by the alpha-chloralose. In the last place, with a percentage of 5, we have herbicides, 40% of the poisoning were caused by amitraz.

A liquid-liquid extraction is conducted for a large chromatographic screening. All the results must be confirmed with a specific search, with the mass m/z for GC-MC and Multi Reaction Monitoring (MRM) with the software library for LC-MS-MS. All the molecules detected, are listed in the table 2.

As an example, figures 11 and 12 show the detection of alpha-chloralose at $t_R = 15.75$ min for GC-MS and at 2.04 min for the LC-MS-MS

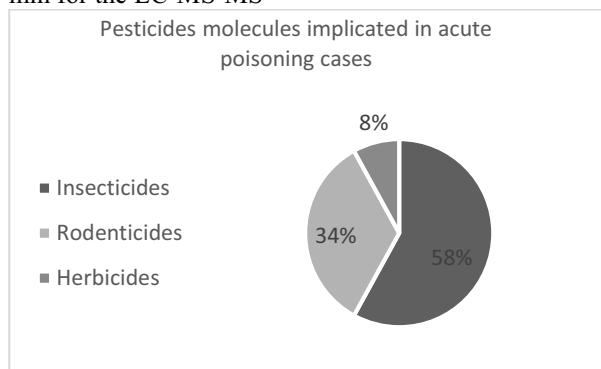


Fig. 10. Pesticides molecules implicated in acute poisoning cases

Table 2. Positive cases poisoned by pesticides

N°	Sex	Age	Pesticides	Orientation and symptoms
1	F	NI*	<i>Carbofuran</i>	NI*
2	F	39	<i>Carbofuran</i>	NI
3	M	52	<i>2,4 d isobutyl ester</i>	Herbicide
4	M	12	<i>Malathion</i>	NI
5	F	NI*	<i>Alphachloralose</i>	Disturbance of conscience
6	M	NI*	<i>Chlorpyrifos, 3,5, 6-trichloropyridinol</i>	Insecticides
7	F	18	<i>3,5, 6-trichloropyridinol</i>	Coma
8	M	NI*	<i>Deltamethrine</i>	Rodenticide
9	F	NI*	<i>Chlorpyrifos</i>	Methomyl
10	M	23	<i>Alphachloralose</i>	Coma
11	F	NI*	<i>Alphachloralose</i>	NI
12	M	2	<i>Chlorpyrifos</i>	Product with diazinon
13	F	65	<i>Alphachloralose</i>	Insecticides
14	F	NI	<i>Malathion, Deltamethrine</i>	Product Igatox
15	F	17	<i>Carbofuran, 3-hydroxycarbofuran</i>	Blue powder – Rodenticide
16	M	NI	<i>Alphachloralose</i>	Rodenticide
17	F	NI	<i>Malathion, Malaxon</i>	Op
18	F	NI	<i>MCPA, 2,4 d, printazole</i>	NI*
19	F	1	<i>Chlorpyrifos</i>	Op
20	M	26	<i>Chlorpyrifos, 3,5, 6-trichloropyridinol</i>	Op
21	M	20	<i>Deltamethrine</i>	NI*
22	F	19	<i>Carbofuran</i>	NI*
23	M	NI*	<i>Deltamethrine</i>	NI*
24	F	NI*	<i>Dichlovos</i>	Op
25	F	NI*	<i>Deltamethrine</i>	NI*
26	M	25	<i>Alphachloralose</i>	NI*
27	F	1	<i>2,4 d isobutyl ester</i>	NI*
28	F	14	<i>Malathion</i>	NI*
29	M	14	<i>Amitraz</i>	Amitraz
30	M	6	<i>Amitraz, Terbutcarb, Diazinon</i>	Hallucination
31	F	3	<i>Terbutcarb</i>	NI*
32	F	3	<i>Dicaraméthyl 2,4 methyl ester, Dicamba</i>	Coma
33	M	NI*	<i>Chlorpyrifos, 3,5, 6-trichloropyridinol</i>	NI*
34	M	21	<i>Alphachloralose</i>	Convulsion
35	F	17	<i>Diazinon</i>	Resperatory distress
36	F	27	<i>Alphachloralose</i>	Rodenticides
37	F	1.5	<i>Bifenthrine</i>	NI*
38	F	56	<i>Malathion</i>	Malathion
39	M	38	<i>Alphachloralose</i>	NI*
40	M	32	<i>3-Hydroxycarofuran</i>	NI*

41	F	50	<i>Dichlorvos, Dimethyl Phosphate</i>	Product Novan
42	M	20	<i>Alphachloralose</i>	Drugs
43	F	NI*	<i>Alphachloralose</i>	Convulsion
44	M	33	<i>Alphachloralose</i>	Ethanol-Méthanol
45	M	19	<i>Alphachloralose</i>	Disturbance of conscience
46	F	14	<i>Alphachloralose</i>	NI*
47	F	NI*	<i>Amitraz</i>	NI*
48	F	17	<i>Alphachloralose</i>	NI*
49	M	40	<i>Alphachloralose</i>	Rodenticides
50	M	NI*	<i>Carbofuran</i>	NI*
51	F	31	<i>Carbofuran, 3-hydroxycarbofuran</i>	Convulsion
52	F	NI*	<i>Alphachloralose</i>	NI*
53	M	NI*	<i>Alphachloralose</i>	Convulsion
54	F	15	<i>Deltamethrine</i>	NI*
55	F	27	<i>Alphachloralose</i>	NI*
56	F	NI*	<i>Alphachloralose</i>	NI*
57	M	4	<i>3,5,6-trichloropyridinol</i>	Convulsion
58	F	NI*	<i>3,5,6-trichloropyridinol</i>	NI*
59	F	2	<i>Carbofuran, 3-hydroxycarbofuran</i>	NI*
60	M	NI*	<i>Chlorpyrifos, 3,5,6-trichloropyridinol</i>	NI
61	F	37	<i>Cypermethrine, Delthamethrine</i>	Pyrethroids
62	F	65	<i>Alphachloralose</i>	Rodenticides and Op
63	F	14	<i>Diazinon, 2-isopropyl-6-methyl-4-pyrimidinol</i>	Insecticides

*NI: Not indicated.

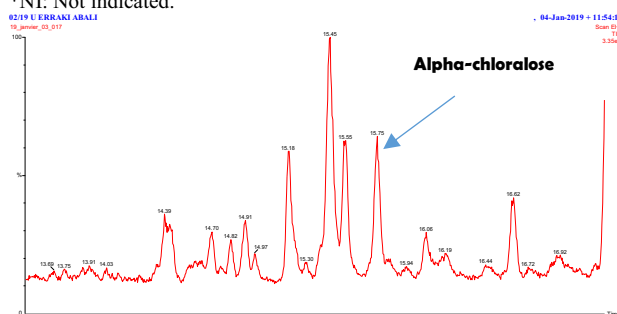


Fig. 11: Detection of Alpha-chloralose by GC-MS

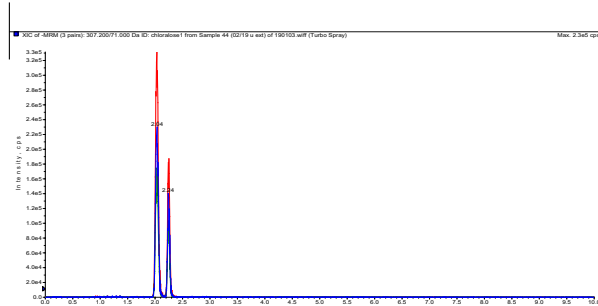


Fig. 12: Detection of Alpha-chloralose by LC-MS-MS

3 Discussion

The acute intoxications by pesticides concern more and more the public health over the world. This study shows the important number of pesticides' poisoning received in our laboratory. Therefore, the Poison Control Centre of Quebec numbered, between 1995 and 2000, 1518 cases related to pesticide intoxication [5]. In France, 408 notifications were received in the Poison Control Centre between 2012 and 2016 [6].

This work concerns 398 cases of acute poisoning by pesticides with 360 chromatographic analyses received from January 2014 to September 2020. The most of these intoxications are accidental especially for children. This result is found also in many other studies [7]. For children under 10 years, the bad emplacement of the domestic products and the non-awareness of the parents leads to the poisoning. In the low-income countries, children are in some cases, obliged to work in the agriculture and this exposure enhances the possibility of poisoning. According to the Achour and al. study [7], the females are more likely to commit suicide with pesticides. This result concurs with ours. We found that 60% of analysis requests are from females and the most of them are a voluntary intoxication.

The insecticides are the first type of pesticides causing poisoning in our study. Many retrospective studies have found similar results [8-9]. These pesticides are provided easily in the market, for domestic consumption in the low-income countries. The rodenticides, herbicides and fungicides are also among the main causes with less percentage representation. For our work, alpha-chloralose was responsible of 33% of the cases. This rodenticide is cheaply sold and chosen for committing suicide. Our results are comparable with other studies [10-12].

Cholinesterase measurement guides the diagnosis to Op or Cbt intoxications. The dosage of the AChE is more reliable than the BChE. There is a correlation between the decrease of the AChE value and the severity of the neuromuscular situation. However, the measurement of the BChE is not consistent because it can decrease naturally with age, sex, during pregnancy, certain pathologies or when taking drugs. For the Op, a specific dosage confirms that the cholinesterase inhibition is caused by pesticides exposition. For carbamates, this inhibition is reversible in 24 hours (except for Aldicarb) [13].

There are some similarities in the symptomatology of the poisoning by these different pesticide families. Muscarinic syndrome, nicotinic syndrome and a central syndrome which convulsions, coma, and respiratory distress, are observed for Op and Cbt poisoning with less pronounced symptoms for Cbt. Pyrethroids induce ataxia and convulsions. In this situation, a cholinesterase measurement excludes the suspicion of Op or Cbt poisoning. Yet, the separative methods decide which the incriminated compound is. It maintains a process to identify the specific patient treatment. Antidote treatment is based on the administration of atropine sulfate and pralidoxime methyl sulfate for Op and Cbt [13].

The CAPM is the only center in the country contributing in the toxicological field adopting the combined

chromatographic analysis. Gas chromatography is recommended for pesticides' analysis for their volatility. The mass spectroscopy detector performs the identification by the mass m/z of the compound with its fragments and a retention time is given according to the temperature program. The liquid chromatography facilitate the separation for thermolabile compounds, with a solvent as mobile phase. For this technic, the mass spectrometry specify the MRMs of the compound according to the library and the retention time (t_R). Combining these two technics for the detection of pesticides, solidify the results transmitted to the hospital to guide the management of patients. The confirmation is operated with chromatographic analysis [14]. This analysis provides additional information about other molecules present in the samples. The multi-systematic method used in this work, detected pesticides in biological sample using a liquid-liquid extraction among 62 patients. Alpha-chloralose was detected in 21 cases, 4 cases only were oriented to this rodenticide and 7 cases had not any indication of the cause of the poisoning. For the alpha-chloralose cases, chromatographic analysis helped to identify the cause of the poisoning. This ascertainment is found in other studies [15-17]. Nevertheless, the negative results is related to the kinetics of the compounds. The timing of the sampling did not help to detect the molecule before the metabolism or the elimination.

Conclusion

The diagnosis and treatment of acute intoxication by pesticides is a big challenge for clinicians and analysts. The toxicological analysis helps to perform correctly the analytical process of any poisoning. An awareness program of the population is important to reduce the accidental intoxication especially among children.

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