INSTITUTO SUPERIOR DE ENGENHARIA DO PORTO
MESTRADO EM ENGENHARIA QUÍMICA




Evaluation of permethrin in rats brains

FRANCESCO VALENTINI
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ABSTRACT

Pyrethrins and pyrethroids interfere with the functions of the nervous system. Exposure to very high levels of these substances can cause dizziness, headaches, nausea, muscle spasms, weakness, loss of consciousness and convulsions. There is no evidence that pyrethrins and pyrethroids can affect the reproductive capacity of human beings but some studies have shown reduced fertility in animals.

The Occupational Safety and Health Administration (OSHA) has established in 5 milligrams of pyrethrins per cubic meter of air over 40 hours of weekly work, the concentration limit of this substance in the workplace.

From this study, it is possible to conclude that is possible to use a methodology to quantify permethrin residues in rats' brains.

It was proved that permethrin can remain in brain during long time. 50% of the rats have permethrin after 24h.

The exposition with alkaline water increases the levels of permethrin in brain. By other side, vitamin E, may act as a protecting factor decreasing the levels until not detected.

Key Words: Permethrin, brains, rats

INTRODUCTION

The pyrethroids are a class of synthetic insecticides and acaroids. Are synthetic analogues of pyrethrins, natural constituents of the flowers of *Tanacetum cinerariifolium* (Fig 1).

Figure 1. 1Flowers of Tanacetum cinerariifolium

Pyrethrum is a toxic insecticide effective, which proves to be a useful ally in the defense strategies of vegetables and fruit in organic production. Pyrethrum is in all likelihood, present in almost all of our homes, at least in summer: we spread it in the air to ward off pesky mosquitoes. The origin of this substance is vegetable the pyrethrum is indeed a flower of the Tanacetum cinerariifolium. Pyrethrum is an insecticide of contact, which acts on the nervous system of insects, preventing them from moving and bringing them to a complete paralysis. However, given its action very persistent, may not result in death of the insect hit.

Due to the similarity of the molecule, they are in fact to act in the same way as the corresponding natural origin, but overcoming the main limitation of pyrethrins: their photolability. We have so much more available active persistent.

The first synthetic pyrethroid, the fenvalerate, was placed on the market in 1978 and today the class consists of 42 active ingredients. Pyrethroids are not able to penetrate into the plant for which exert action predominantly by contact, favored by their lipid solubility that allows the penetration into the epicuticular waxes.

Their mechanism of action is based on the interaction stereoselective of sodium channels which causes a prolongation of the flow of sodium during the excitation. Can cause abnormal sensations in the face rather frequent (30 to 70 % of complaints), appear after a few minutes up to an hour

after exposure and last for no more than 24 hours; sometimes they are confused with allergic reactions.

It is a non-selective insecticide that was indiscriminately both harmful insects that those profits, so should be used with caution.

On the other hand, it is a substance that degrades quickly and easily with the exposure to temperature and sunlight, for which exerts its action in a period of time rather narrow.
At the same time, it is not 'systemic' (is not able to enter into circulation in the fluids of plants), nor "cyto- tropic" (that is not able to through tissues).
It is considered an insecticide to all the effects, despite its natural origin, since it is a substance that strikes indiscriminately insects with which it comes into contact.
Pyrethrum is just little toxicity towards warm-blooded animals mammals and birds), but instead highly toxic to fish, reptiles and amphibians.

Biological effects:

The primary site of action of both classes, it is at the level of sodium channels at the level of the cell membrane of the excitable elements.
After the passage of an action potential in excitable cells of the nervous tissue and of that muscular, the **pyrethrine** and **pyrethroid** blocking the opening of a certain percentage of membrane channels for sodium. These agents are then known blockers the opening of the channels. Normally, after the passage of an action potential, the initial flow of sodium ions into the interior of the cell it is rapidly reduced.
In the presence of **pyrethrins** and **pyrethroids**, instead, it has the persistence of a certain influx of sodium. The continuous influx of sodium ions causes, after the primary action potential, a prolongation of the current, this, at the same time, It is the cause to repeated shocks of excitable cells.
The persistent, this depolarization inhibits the propagation of further action potentials, causing, therefore, nerve palsy.(1)
The primary site of action of both classes is at the level of sodium channels in the cell membrane of excitable elements. After the passage of an action potential in excitable cells of the nervous and muscular tissue, pyrethrins and pyrethroids block the opening of a certain percentage of

membrane channels for sodium. These agents are then known blockers of the opening of channels.

Normally, after the passage of an action potential, the initial flow of sodium ions into the interior of the cell is rapidly reduced. In the presence of pyrethrins and pyrethroids, instead, there is the continuation of a certain influx of sodium. The continuous influx of sodium ions determines after the action potential prolongation of the primary current. This, in turn, is due to repeated discharges of excitable cells. The persistence of this depolarization inhibits the propagation of further action potentials, causing, therefore, nerve palsy. (2)

Pesticides and autism:

The Davis Mind Institute of the University of California, Sacramento has published a study in which it is shown the relationship between the exposure within a radius of 1.5 km, in pesticides and in human fetuses onset of autism and developmental delays. The study considers three classes of widely used pesticides: organophosphates, pyrethroids and carbamates. The results are chilling, and retrace as established by EFSA in January, in relation to some active ingredients of neonicotinoids. The use of these substances also not in the immediate vicinity of a pregnant woman can cause delays in neurological development of fetuses and even autism. Out of 970 study participants, 486 cases have seen the onset of autism, developmental delays and 168 cases only 316 children born with no problem. The onset of autism was associated especially with the use of organophosphates, among them the chlorpyrifos. Not least the effects of pyrethroids, widely used in our towns for their anti-mosquitoes. Cypermethrin, permethrin, esfenvalerate are all active indicted. The study confirms it: "The children of mothers residing near (range 1.5 km) to places of applications of insecticides based on pyrethroids is just before conception and during the 3rd quarter were exposed to a greater risk for both autism that the delay in the development. " It will be high time to ban forever even the fearsome pyrethroids that starting from May, ending in October, they are indiscriminately sprayed in areas not far kilometers from our homes, but in our homes to fight the development of adult mosquitoes? The study by the University of California once again the links between diseases related to neurological development of newborns of mothers and exposure to pesticides. Once again, the cry of alarm of beekeepers around the world

reveals a tragic warning to all humanity subjugated by an increasingly delusional chemical industry.(3)

According to an American study, pregnant women proximity to toxins increases the risk of autism and retardation for the baby. The warning comes from the scholarly researcher Irva Hertz Picciotto UC Davis Mind Institute of California, a lecturer at the Department of Public Health Sciences at the same institution. According to the findings from the studies of the American researcher, if a pregnant woman comes into contact with pesticides, the risk of autism and mental retardation for the unborn increases significantly.

The study conducted by a team of researchers at the University of California at Davis, published in the journal Environmental Health Perspectives revealed that the strongest associations were registered when the exposure had occurred during the second and third trimesters of pregnancy. The researchers examined the associations between specific classes of pesticides, including organophosphates, pyrethroids and carbamates, which the women were exposed during pregnancy, and later diagnosis of autism and developmental delay in children.

Behind the correlation between increased risk of autism infant and contact with pesticides mothers, there would be the poisonous effect caused in the body of pregnant women from dangerous organophosphates such as acephate, chlorpyrifos and diazinon. In particular, chlorpyrifos would cause the most severe damage especially if the exposure happens in the third month of pregnancy.

For their part, other pesticides such as pyrethroids esfenvalerate, lambda-cyhalothrin, permethrin, cypermethrin and tau-fluvalinate seem to play a significant role in the incidence of autism on the unborn child and the mental retardation seems to be more stimulated by contact with carbamates as methomyl and carbaryl.

As determined by scientific research, pesticides have neurotoxic implications, and consequences that can directly affect the proper development of the brain of the fetus during pregnancy. Exposure to this type of toxic substances by pregnant women, which can cause abnormalities in cognitive mechanisms of the baby, causing disturbances in the degree of impact on brain areas that regulate mood, social interaction, behavior and learning child. (4)

This study will be focused in a pyrethroid half-life, the permethrin, in rats brains. A special detail is given about this pyrethoid.

PERMETHRIN ($C_{21}H_{20}Cl_2O_3$) acts as a neurotoxin. It is in natural form a liquid yellowish brownish, which tends to crystallize at room temperature. The melting point is 34-39°C. (4)
It is an active substance pesticide used for medical-surgical aids and as an insecticide.
In agriculture it is mainly used to fumigate crops of cotton, wheat, corn and medical use while on farms located in killing the parasites in chickens. Its use is controversial because, being a broad-spectrum poison, killing indiscriminately the various species of insects including those not harmful like bees.
It is used to eliminate human parasites such as lice and scabies and to control cockroaches, termites, ants both in domestic and industrial environments. (5)

Permethrin eliminates mites and ticks to the simple contact with treated fabrics. According to studies of the Connecticut Department of Public Health has a low mammalian toxicity, absorption through the skin is limited and allergic reactions are rare. It is, therefore, also used on dogs to eliminate the same pests and to keep away mosquitoes and sandflies responsible for the transmission of leishmaniasis. Permethrin must never be used on cats, as they can easily cause death through the airways. This is because cats do not have the enzymes necessary to eliminate the molecule of permethrin from their body, behaving like a real poison. The products "spot-on" containing permethrin to be used on dogs, guarantee the absence of mosquitoes and sandflies for only two weeks, so it must be applied more frequently during the spring and summer months.(6)

-To go where the mosquito-borne diseases are endemic must equip themselves with insecticides and repellents to be applied on clothing and mosquito nets. There are also milder solutions to use at home.

Permethrin is a pyrethroid with a very low toxicity to mammals. Absorption through the skin is limited and only rarely is believed to be responsible for allergic reactions. It is also effective for a wide range of pests, for this is present in numerous products, from the flea to animals, for people

with lice and mosquito sprays commonly used in family. It is already used in agriculture: approved by the Environmental Protection Agency, for decades is a method of pest control in various crops.

When traveling in tropical countries or places where the mosquito-borne diseases are endemic should be used more effective methods of protection in order to avoid being infected. Malaria is not just a concern, coma diseases dengue and West Nile virus is spreading more and more severely affecting Brazil, India, Thailand and even the island of Madeira, where an epidemic of Dengue in infected many people in the summer of 2012.
While traveling for business or fun to be you must protect themselves. To do this, the World Health Organization recommended to protect clothing, and spraying mosquito repellent with permethrin.(7)

Permethrin to fabric is usually available in spray form. A spray bottle that 100/150 grams may be sufficient to treat two changes of garments. Permethrin applied appropriately resin extract, and the clothes are soaked up to 8 weeks. The mosquito nets should be treated monthly to maintain their effectiveness.

CHAPTER 2: Methodology- Background

Gas Chromatography

The chromatograph consists of an injector, a system for controlling the temperature of the column and a transfer line which allows the effluent of the column to enter the mass spectrometer. Gas Chromatography (GC or GLC) is a commonly used analytic technique in many research and industrial laboratories for quality control as well as identification and quantification of compounds in a mixture. GC is also a frequently used technique in many environmental and forensic laboratories because it permits the detection of very small quantities. A broad variety of samples can be analysed as long as the compounds have sufficient thermal stability and are volatile. A mobile and a stationary phase are required for this technique. The mobile phase (carrier gas) is comprised of an inert gas i.e., helium, argon, or nitrogen. The stationary phase consists of a packed column where the packing or solid support itself acts as stationary phase, or is coated with the liquid stationary phase (high boiling polymer). Most analytical gas chromatographs use capillary columns, where the stationary phase coats the walls of a small-diameter tube directly (i.e., 0.25 µm film in a 0.32 mm tube). The separation of compounds is based on the different strengths of interaction of the compounds with the stationary phase ("like-dissolves-like"-rule). The stronger the interaction is, the longer the compound interacts with the stationary phase and more time it takes to migrate through the column (=longer retention time).(8)

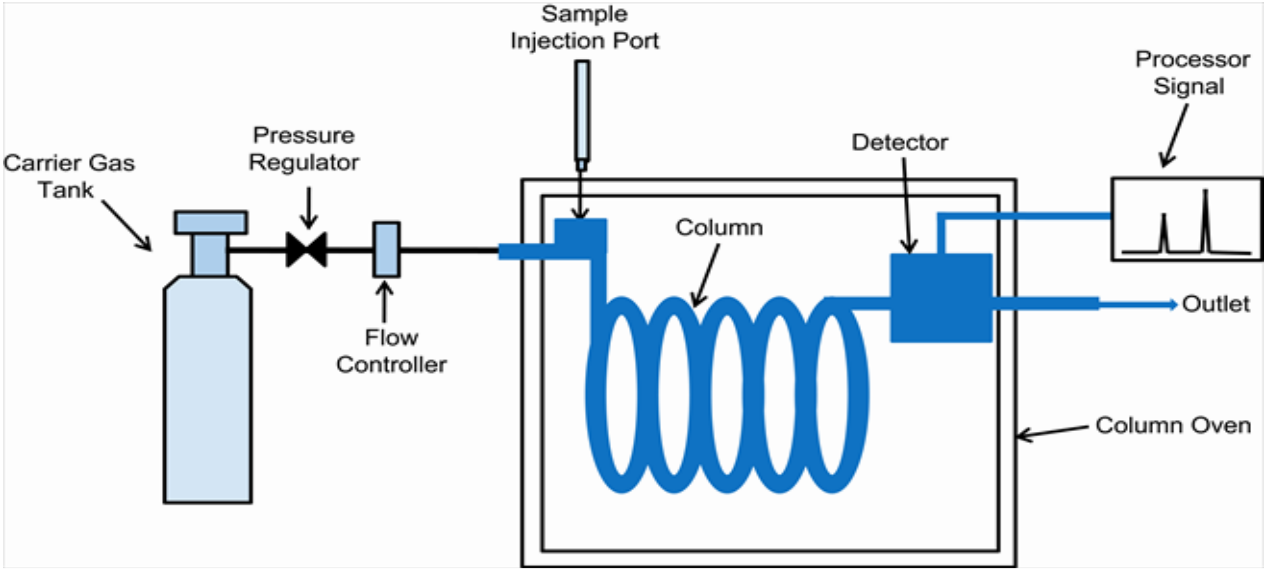

Figure2.1 general GC-equipment

The factors which influence the separation of the components are:

- Boiling point - The boiling point of a compound is often related to its polarity. The lower the boiling point is the shorter the retention time is because the compound will spend more time in the gas phase. That is one of the main reasons why low boiling solvents (i.e., diethyl ether, dichloromethane) are used as solvents to dissolve the sample. The temperature of the column does not have to be above the boiling point because every compound has a non-zero vapour pressure at any given temperature.
- The polarity of components versus the polarity of stationary phase on the column.

If the polarity of the stationary phase and compound are similar, the retention time increases because the compound interacts more strongly with the stationary phase. As a result, polar compounds have long retention times on polar stationary phases and shorter retention times on non-polar columns using the same temperature.

- Column temperature, an excessively high column temperature results in very short retention time but also in a very poor separation because all components mainly stay in the gas phase. If the compound does not interact with the stationary phase, the retention time will decrease. At the same time, the quality of the separation deteriorates, because the differences in retention times are not as pronounced anymore.

- Carrier gas flow rate, a high flow rate reduces retention time, however, a poor separation should be observed as well. As above, the components have very little time to interact with the stationary phase and are just being pushed through the column.(5)
- Column length a longer column generally improves the separation. The trade-off is that the retention time increases proportionally to the column length and a significant peak broadening will be observed as well because of increased longitudinal diffusion inside the column.
- Amount of material injected ideally, the peaks in the chromatogram display a symmetric shape (Gaussian curve). If too much of the sample is injected, the peaks show a significant tailing, which causes a poorer separation. Most detectors are relatively sensitive and do not need a lot of material in order to produce a detectable signal.

GC-ECD (Gas chromatography Electron capture detectors)

In an electron capture detector, (ECD) a radioisotope, usually ^{63}Ni deposited on a foil of gold, is used as the source. These emits β rays or fast electrons that ionize the carrier gas, producing slow electrons and positive ions that give us a certain value of current. In the case in which the carrier gas is nitrogen the ionization reaction is N_2 + particle β → N2 + + slow electrons.(9)

In fact, the radioactive foil constitutes the cathode of an electrical circuit: when the ions are generated these go to close the circuit, producing an electrical signal in terms of potential difference or current intensity. Compounds containing electronegative atoms, strongly absorbing the flow of slow electrons between the source and a detector of the electrons, can be detected by the way with streaming from the gas chromatography column (10). In fact these molecules capture the slow electrons that are generated by the ionization of the carrier gas, and then go to reduce the background current which is usually generated as these are of lesser mobility. The peaks consequently are directed downwards because they involve a decrease of the current, and this decrease will be proportional to the quantity of ions produced, and then the concentration of the substance present in the sample. Typically these molecules would be hardly noticeable with other detectors, for example many halogenated compounds in addition to not burn are even extinguishing the flame, and would pose problems to a FID. The most consistent defect lies in the fact that the capacity of the molecules to capture slow electrons depends on the energy of the

same, and therefore from the applied potential; the sensitivity and selectivity depend therefore from the latter parameter.(11)

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CHAPTER 3: Experimental Part:

3 Material e Methods

3.1.Reagents and materials

Permethrin was purchased from FLUKA establishments and has a degree of purity equal to 95.7 %, n-Hexane come from VWR Prolabo CHEMICALS (France Rue carnot201, Fontenain-sous) It has a purity of 99,6%.

The Falcon conical (50 mL) used were from VWR International, Vial 1,5mL came from SPECANALITICA (Great Britain), insert 0,2 mL came from BGB (Germany), Pipette manual adjustable came from VWR (North America), Cleanup is ENVIRO CLEAN from UCT (Bartram, Bristol, PA) 15O mg MgSO4, 15Omg CUPSA, 50 mg CEC 18.

3.2 **Equipments**

An ANALOG VORTEX MIXER from VWR and an ULTRASOUNDS SONOROX were used to homogenize solutions. A CENTRIFUGE 2-16 SARTORIUS was used to separate phases. A homogenizer mechanical with ultrasounds to liquefy samples and GC-ECD from Shimadzu to quantify permethrin were used.

3.2. Sample preparation

3.2.1 Homogenization of rat brains:

With the aid of a drill of the hospital's medical department S. João of Porto city, the homogenization of the entire samples of rat brain were achieved with, hexane as a solvent. The procedure was as follow: 1) the rat brains was contained within enclosures - 18 ° to avoid degradation; 2) took the brains and homogenized several minutes with the drill on ice to keep the

temperature low; 3) took the homogenized and placed in a falcon and put inside a Styrofoam container to prevent heat loss during the journey from hospital S. João to ISEP.

3.2.2 Permethrin extraction from brain homogenate:

Liquid liquid extraction (LLE) is one of the most widely employed and useful techniques in Pharmaceutical sample preparation. This is due to a number of characteristics, including simplicity, rapid method development, and reasonable selectivity.

LLE involves adding a solvent to the sample that is immiscible, followed by selective partitioning of analytes versus contaminants between the two phases.
In the interest of extraction completeness, it is necessary to use an adequate amount of extracting solvent to capture all of the analytes from the original sample.
This extraction solvent is added to the sample, then the two phases are mixed, by a vortex) or shaking, to bring about substantial physical mixing. After agitation the phases are allowed to separate.

The first step of extraction consists of mixing hexane and rat brain thanks aid of vortex for 5 ', second step after this is take the mix contained in a falcon in the ultrasound equipment and will you leave to a temperature of 21 °C for 15 ', the third step is to bring the falcon in the centrifuge equipment to work at 4000 rpm for the duration of 10'.

At this point we will have the separation of the phases, where the brain (heavier than hexane) will be located in the lower part and the hexane containing the permethrin extracted from the brain, other ones are in the upper part, at this point with the help of a pipette take all the hexane and the permethrin for storing the liquid, about 2 mL, in a 10 mL flask. This operation will be repeated 3 times.

Once having repeated for three times the LLE, it was achieved a volume of approximately 6 mL of suspension.

After that we place the flask containing the suspension under a gentle flow of nitrogen (N_2) to evaporate throughout the hexane contained in the flask.

Finally, 1500 µL of n-hexane was added to dissolve the residue, the resulting solution was shaken vigorously using a vortex device and the extract was then placed into an inside the vial, ready for be injected in the GC-ECD.

A cleanup step was performed with cleanup (Enviro Clean comprising 150 mg MgSO4) to prevent damage and be sure that the matrix components are retained but the analyte of interest (Permethrin) is not.

3.3. Analytical methodology for permethrin quantification

Permethrin was analyzed using a Shimadzu GC-2010 with ECD apparatus equipped with a capillary column ZB-XLB (30 m x 0.25 mm x 0, 25 mm) from Phenomenex. A volume of injection 1µL was used. The oven temperature was programmed starting at 60°C and held for 1'min followed by increase of 30°C/min to 250°C and then increase 10°C/min until 290°C and held for 3'. The detection was 300°C. Helium (Linde Sogas) was used as carrier gas at a constant flow rate of 1.3 mL/min, whereas nitrogen (Linde Sogas, purity 99.999%) was employed as makeup gas at a flow of 30 mL/min. The system was operated by GCsolution Shimadzu software.

CHAPTER 4 : RESULT AND DISCUSSION:

4.1 Validation parameters

To be sure to observe the peaks of permethrin provided by GC-ECD we made a preliminary chromatogram analyzing only permethrin and hexane into the GC-ECD.

4.1.1 Retention time

The range of the two peaks of permethrin is from 21.5 to 22.5 (Fig 5.1).:

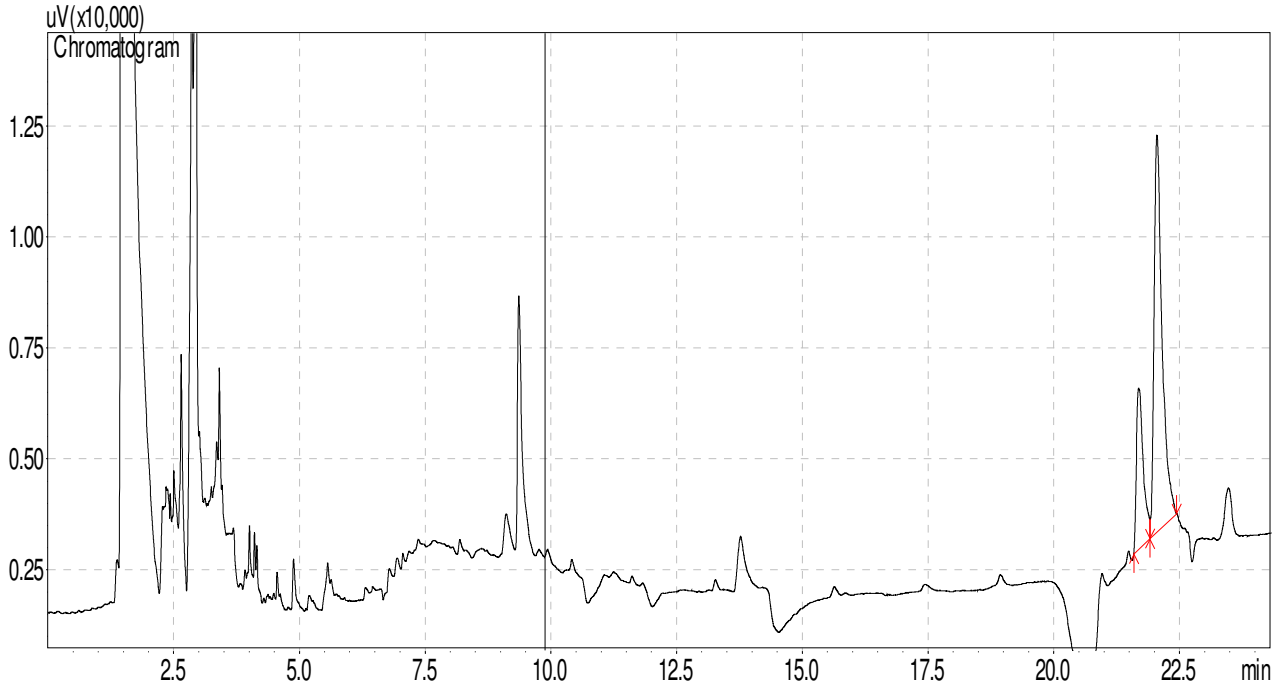

Figure 4.1 ECD chromatogram of permethin solution at 20µg/L

4.1.2. Linearity and calibration curve

A calibration curve was obtained for permethrin, by injections in triplicate of standards at six calibration levels (Fig 5.2 and Fig 5.3).

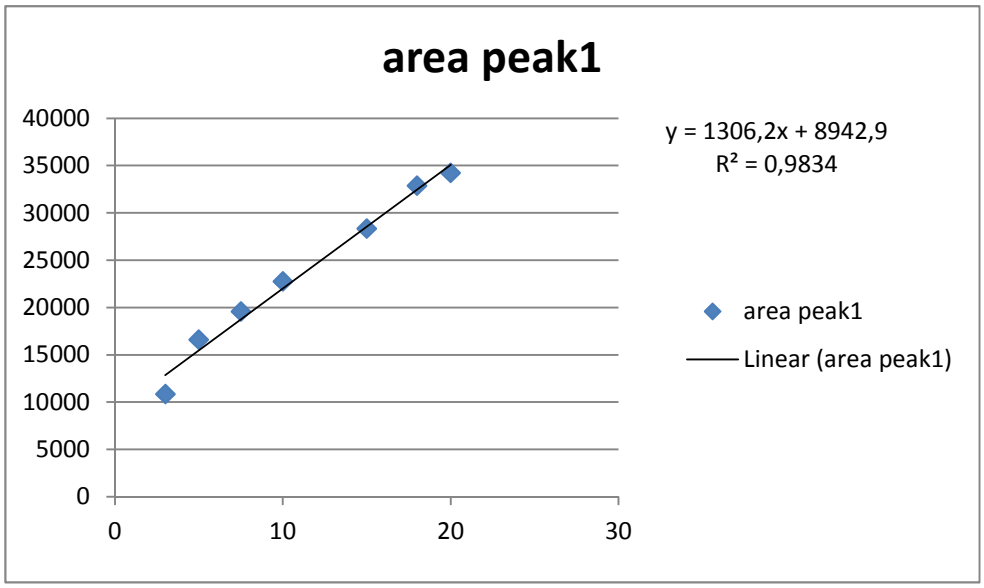

Figure 4.2 Calibration curve of the first peak of permethrin (Area vs permethrin µg/L)

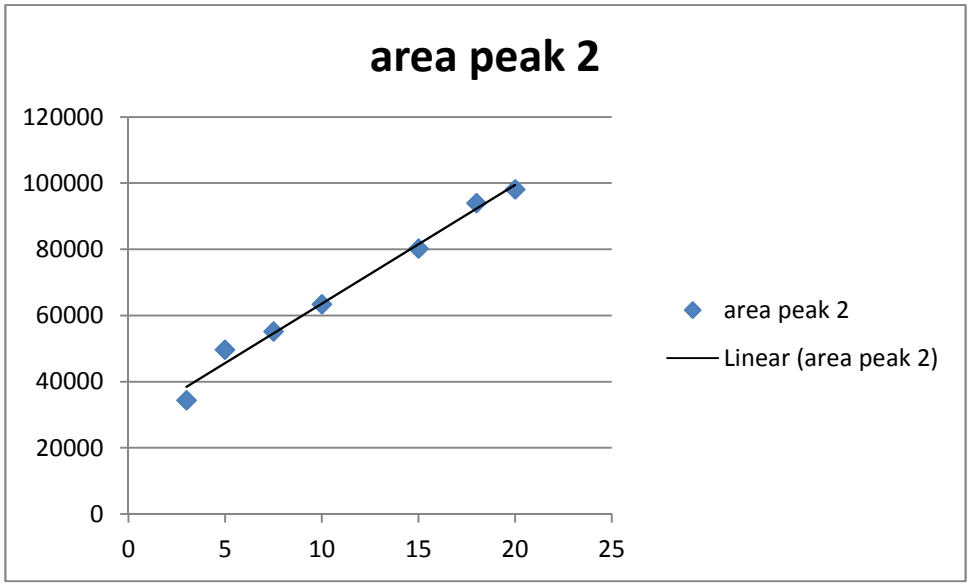

Figure 4.3 Calibration curve of the second peak of permethrin (Area vs permethrin µg/L)

After receiving the results of the calibration curves, we could move on to analyze the brains of control rats that had not been treated her with permethrin. At this stage we gradually added amount of permethrin. The calibration curves of the peaks of permethrin in the matrix were performed.

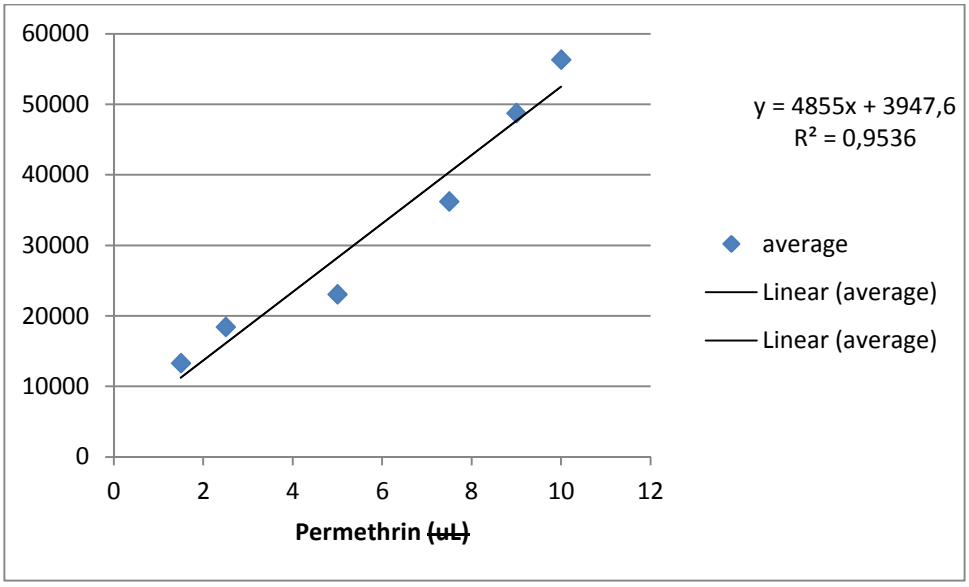

Figure 4.4 Calibration curve of the first peak of permethrin in brain (Area vs permethrin µg/brain)

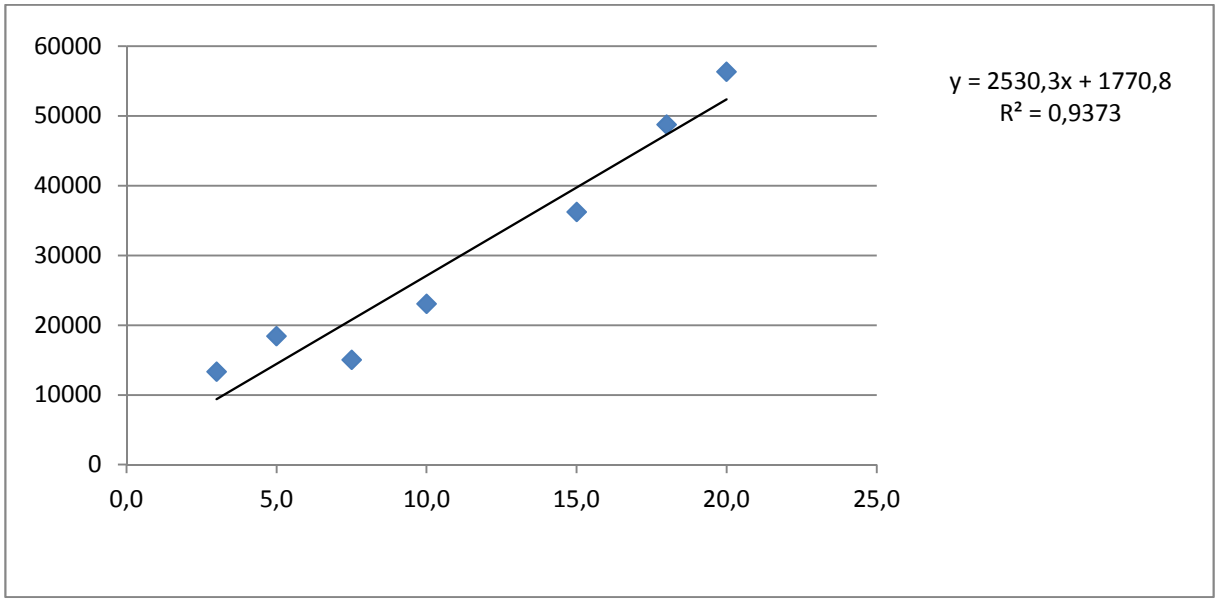

Figure 4 .5. Calibration curve of the second peak of permethrin in brain (Area vs permethrin µg/brain)

4.1.3. Recovery evaluation

To test the procedure a control brain was spiked with an amount of permethrin. The recovery was obtained by the ratio area of permethrin in the brain vs area of permethrin in the solvent.

$$RECOVERIES = \frac{A2}{A1} \qquad \frac{62371,5}{65459,9} = 0,953$$

A_2 : (average area control brain spiked with 20 µL of permethrin)

A_1 : (average area of 20 µL of calibration curve)

4.2 Samples analysis

After these preliminary studies it was switched to the study of permethrin inside the brains of rats that had been provided. Analyzing, if inside brain there was the presence of permethrin or if it had been completely metabolized.

The Table 5.1 shows the achieved permethrin area, concentration applying the matrix calibration curve and the amount of permethrin per rat brain.

All control brains didn´t achieved any detectable amount of permethrin.

However the rats treated with permethrin only 40% revealed detected permethrin (2 samples with 0,008 and 0,003 µg/brain). The rats 60 days old any of them achieved permethrin above the limit of detection. The rats (22 days old) 24h after treatment reveals that 50% has permethrin in the brain between 0,006 and 0,008 µg/brain.

Comparing the rats brains, exposed to permethrin with alkaline water and vitamin E, it seems that vitamin E is a protecting factor to permethrin exposure. None of the brains exposed to permethrin

and vitamin E revealed permethrin. Although the brains exposed with alkaline water present permethrin between 0,0013 and 0,0093 µg/brain.

Table 4.1. Analyzed samples and amount of permethrin achieved per brain

SAMPLE	Area	Concentration (with matrix calibration curve)	Permethrin (µg/brain)
Control brain I	nd	nd	nd
Control brain II	nd	nd	nd
Control brain II	nd	nd	nd
Control brain IV	nd	nd	nd
Control brain V	nd	nd	nd
CONTROL (60 days old)	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
Treated Brain	63.446,2	15,86	0,008
	27.004,7	6,75	0,0026
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
PERMETHRIN (60 days old)	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
Rat brain male, 24h after treatment (22 days old)	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	51.753,3	12,93	0,0062
	60.097,2	15,01	0,0075
	52.227	13,05	0,0063
PERMETHRIN+ALC. (60 days old)	72319,65	18,07	0,0093
	34302,25	8,57	0,0037
	26436,50	6,60	0,0026
	54564,32	13,63	0,0067
	17487,80	4,37	0,0013
	13124,70	3,28	0,0006
	10941,40	2,73	0,0003
	62826,40	15,7	0,0080
	15482,50	3,87	0,0010
	43658,76	10,91	0,0052

PERMETHRIN+Vit.E (60 days old)	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd

nd- not detected

CHAPTER 5: CONCLUSIONS

From this study, it is possible to conclude that is possible to use a methodology to quantify permethrin residues in rats' brains.

It was proved that permethrin can remain in brain during long time. 50% of the rats have permethrin after 24h.

The exposition with alkaline water increases the levels of permethrin in brain. By other side, vitamin E, may act as a protecting factor decreasing the levels until not detected.

REFERENCIES

1) *Source: http://www.infozanzare.info/prodottichimici.php (Accessed in January of 2016)*

2) Source: http://www.janssen-italia.it/news/Gravidanza-e-pesticidi-bambini-a-rischio-autismo%20 (Accessed in January of 2016)

3) "Library and Archive Catalogue EC/1974/16: Lovelock, James Ephraim". London: The Royal Society. Archived from *the original* on 2014-04-10.

4) Zhou, T., Zhou, W.,Wang, Q., Dai, P.-L,F.,Zhang, Y.-L.,Sun, J.-H.,2001. Effect of pyrethroids on neuronal excitability. Pesticide Biochemistry and Phisiology 100, 35-40.

5) Dong,K.,2007. Insect sodium channel and insecticide resistance. Invert Neuroci 7, 17-30

6) Source: http://www.infozanzare.info/prodottichimici.php *(Accessed in January of 2016)*

7) Dong,K.,2007. Insect sodiumchanneland insecticide resistance. Invert Neuroci 7, 17-30

8) Lovelock, J. E. (1958). "A sensitive detector for gas chromatography". Journal of Chromatography A 1: 35.
doi:*10.1016/S0021-9673(00)93398-3*.

9) Lovelock, J. E. (1974). "The electron capture detector". Journal of Chromatography A 99: 3.
doi:*10.1016/S0021-9673(00)90840-9*.

*10) Jump up^* Krejči, M.; Dressler, M. (1970). "Selective detectors in gas chromatography". Chromatographic Reviews: 1.

doi:*10.1016/0009-5907(70)80005-9*. *(Accessed in January of 2016)*

*11) Jump up^* Krejči, M.; Dressler, M. (1970). "Selective detectors in gas chromatography". Chromatographic Reviews: 1.
doi:*10.1016/00095-9*.

12) Agency for Toxic Substances and Disease Registry (ATSDR). 2003. Toxicological Profile for Pyrethrins and Pyrethroids. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

APPENDIX:

Chemical and physical properties:

Common name : permethrin

Chemical name : 3-fenossibenzil (1RS) cis, trans-3-(2,2-diclorovinil)-2,2- di-metil-ciclopropan-carbossilate .

Empirical formula: $C_{21}H_{20}Cl_2O_3$

Molecular weight : 391,3

Solubility in water : 0,2 mg/l a 25 °C

Form : Liquid yellowish brownish , which tends to crystallize at room temperature

Melting point : 34-39 ° C (pure permethrin) , 63-65 ° C for the cis isomer , trans isomer 44-47 ° C

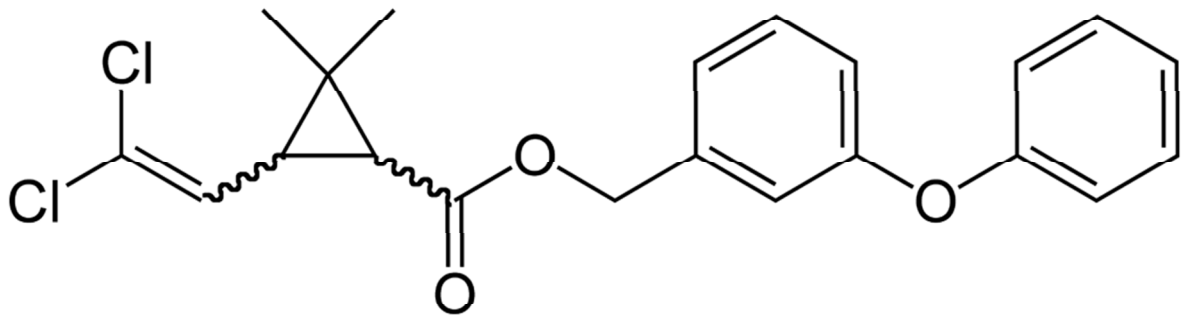

Figure A.1. Chemical structure of permethrin

Calibration curve of permethrin and Hexane by GC-ECD Shimadzu:

Table A1 Results of calibration curve obtained of permethrin between 3 to 20 µg/L permethrin.

ug/L permethrin	repeat 1	repat 2	repeat 3	avarage
3	11632	11239,9	9681,9	11435,95
	33810,1	37000	32496,4	34435,5
5	17810,6	16537,2	15416,6	16588,1333
	55039,5	52546,7	41630	49738,7333
7,5	19504,4	18382,2	20818	19568,2
	56113,7	51800,9	57649,5	55188,0333
10	21796,4	22304,5	24103,1	22734,6667
	60543,3	60241	69499,3	63427,8667
15	24103,1	27908,2	33001,2	28337,5
	69499,3	77660,2	93652,3	80270,6
18	32290,4	30809,1	35460,6	32853,3667
	90209,6	91860,4	99806,6	93958,8667
20	33638,1	34485,7	34489,8	34204,5333
	97766,5	98708,7	97843	98106,0667

Table A2 Results of calibration curve obtained of permethrin between 5 to 30 µg/L permethrin.

ug/L permethrin	repeat 1	repeat 2	repeat 3	Avarage
5	15550,00	15032,5	16736,5	15773,00
	47193,00	44983,6	51248,7	47808,43
10	24992,90	22226,6	19438,2	22219,23
	68722,20	62797,1	62635,2	64718,17
15	31108,70	27799,1	35734,2	31547,33
	87124,40	79766,6	99145,2	88678,73
20	52564,95	56872,1	49473,5	52970,18
	151729,20	271422,9	175768,8	199640,30
25	74021,20	85944,9	88854,6	82940,23
	216334,50	216052,6	245850,8	226079,30
30	105046,90	92888,7	106152,8	101362,80
	259268,90	256037,2	283766,3	266357,47

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“Every day of life is unique, but we need something to happen there touches to remind us. It does not matter if we get the results or not, if we look good or not, after all the essential accounts for most of us, is something that is not seen, but is felt in the heart”.

(Haruki Murakami)