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&

16th Symposium on Chemistry and Fate of Modern Pesticides

joined to

10th MGPR International Symposium of Pesticides in Food and the Environment in Mediterranean Countries:

CONCERNS, CHALLENGES & POSSIBLE SOLUTIONS

LC-MS/MS ASSESSMENT OF FIPRONIL RESIDUES IN CHICKEN EGGS

Alberto Angioni¹, Alessandro Atzei¹, Carla Lai¹, Giulia Pes¹, Eleonora Cossu¹, Maria Teresa Russo²

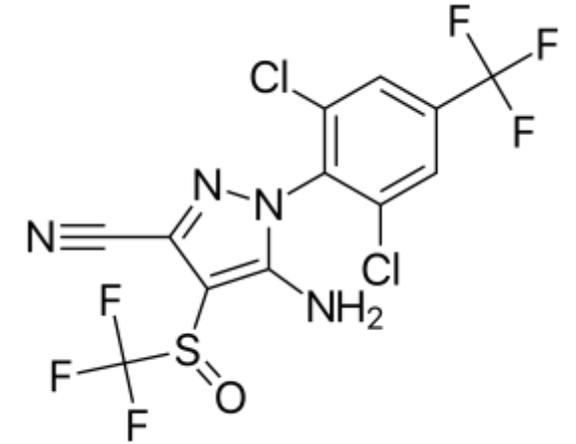
¹*Department of Life and Environmental science University of Cagliari, Cagliari (CA, Italy)*

²*University Mediterranea of Reggio Calabria (RC, Italy)*

corresponding author: aangioni@unica.it

Fipronil

- Fluocyanobenpyrazole, better known with the market name of **Fipronil** is an insecticide widely used in homes and yards to control a variety of pet pest like ticks, fleas, and mosquitoes, and for professional use in agriculture practice.
- Fipronil cannot be used in the control of pest in animals intended for the food chain due to its high toxicity.
- The MRL set for Fipronil is **0,005 mg/kg**, which correspond to the analytical limit of quantification.

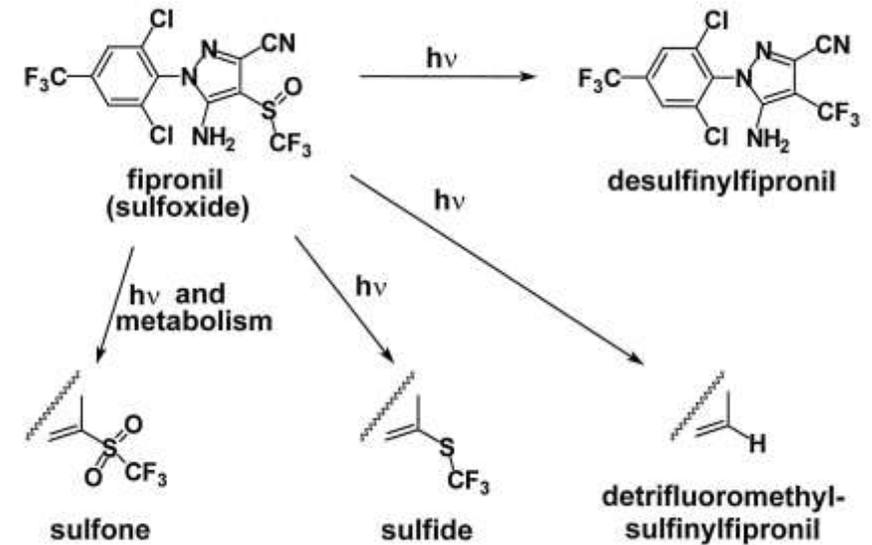
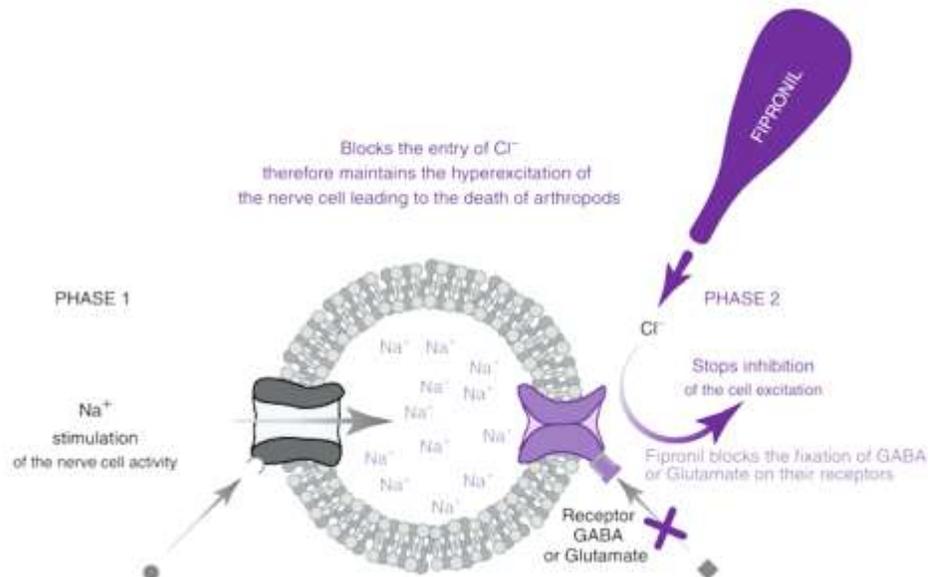
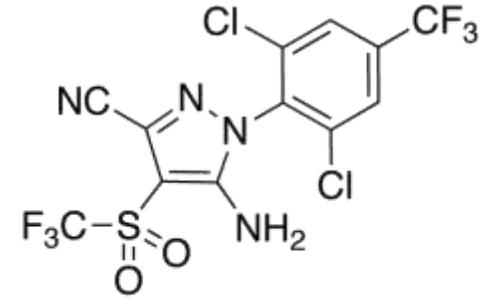


Code number	Products to which MRLs apply (Part A of Annex I to Reg. (EC)/2003)	Maximum Residue Level (mg/kg)
120000	Fipronil (active substance) + sulfone isothiazole (984912) expressed as Fipronil (7) (A)	0.005

EU - Pesticides database

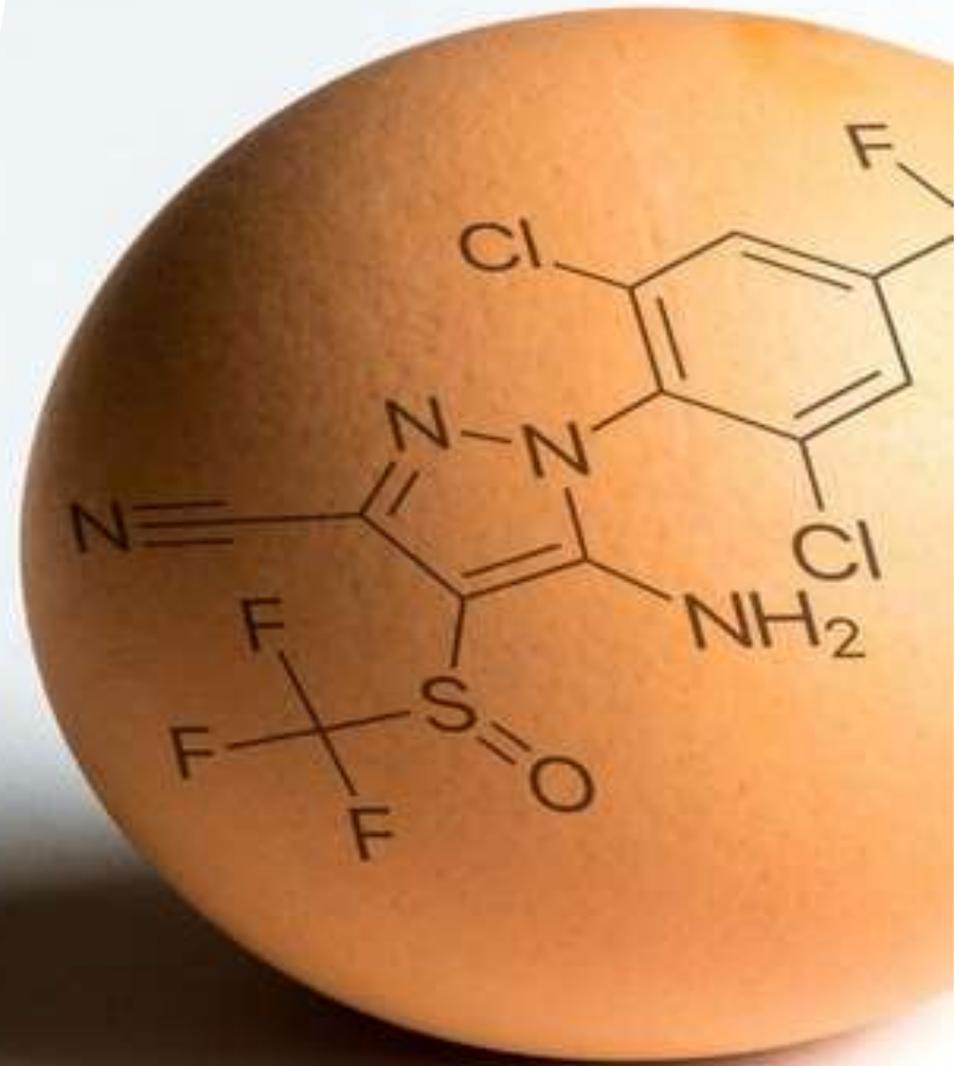
Biochemistry and mechanism of action

- Fipronil is quickly metabolized to environmental level, in vegetables and in animals.
- The major metabolite is the **Fipronil sulfone** (oxidation of the sulphur).
- Fipronil blocks the fixation of GABA or Glutamate on the receptors of the insects cells.
- The entry of Cl^- to the cell is block, therefore maintains the hyperexcitation of the nerve cell leading to the death of arthropods.



Fipronil Toxicity

- * Fipronil is moderately considered toxic and the assumption of around **0,009 mg/kg** body weight per day is esteemed as harmless.
- * A person of 60 kg introduces a reasonable limit of assumption of fipronil of around **0,54 mg/die**.



Backstory

- * Fipronil has gained a high **mediatic attention** after its detection in chicken eggs in concentration higher than the MRL (maximum residue level) approved by the EU.



- * **RASFF** (Food and Feed Safety Alerts) portal shared a notification about fipronil concentration (**0.04 mg/Kg**) detected in eggs from Italy.

RASFF Portal

Notifications list

Classification	Date of case	Reference	Notifying country	Subject	Product Category	Type	Risk decision
INF	06/11/2017	2017-1849	Netherlands	Leakage of beer from tins	alcoholic beverages	food	serious
information for attention	06/11/2017	2017-1848	Italy	Report 0.04 mg/kg - fipronil in eggs from Italy	eggs and egg products	feed	not serious

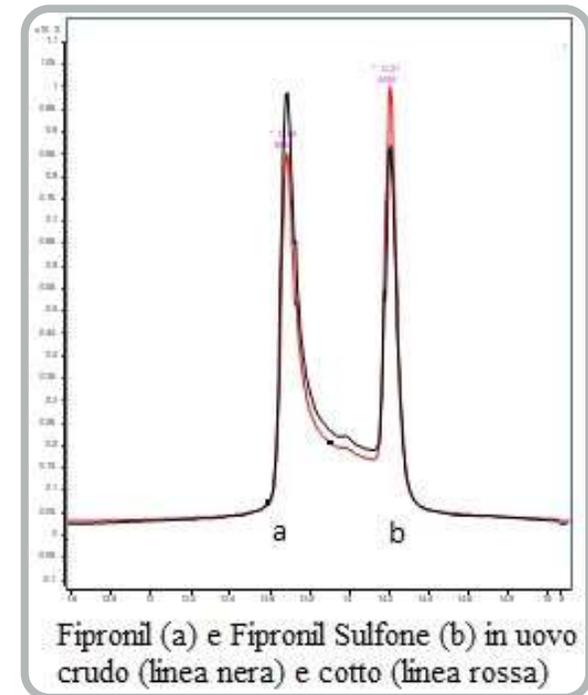
- * Our data showed a high contamination of the eggs from **contaminated chickens (Table 1)** at levels higher than those accepted from EU regulations.
- * The major part of fipronil was degraded in fipronil sulfone, accounting for more of the 99% of the residue.

sample	Fipronil	Fipronil Sulfone
	mg/kg ± RSD%	
MRM	435 > 330	451 > 415
Test 1	0.003 ± 27.52	2.083 ± 7.25
Test 2	0.003 ± 10.38	2.075 ± 4.06
Test 3	0.003 ± 1.37	2.155 ± 1.30

Table 1. Fipronil contamination in chicken eggs from industrial production

- * The analysis carried out highlighted the presence of Fipronil and Fipronil sulfone contamination in all the samples analyzed in concentrations higher than those permitted by law.
- * It also allowed to highlight how it was fundamental to carry out the analysis on both molecules.
- * In fact, the Fipronil levels in all the eggs analyzed showed a mean residual of about 0.003 mg / kg, well below the legal limits, while the fipronil sulfone showed residues of about **2 mg / kg**.

- Moreover, it was shown that cooking did not decrease the levels of fipronil (a) and fipronil sulfone (b) residues in eggs.



What is the origin?



- * The origin of the contamination is still **unknown**, and several hypotheses have been made:
 - an illegal use on the young chicken before their arrival in the eggs producing farms.
 - an illegal treatment of the feed.

But till today the real origin has not been revealed.

Residues has been also detected in farms far from each other, and with no connection among them.

- * Therefore, a monitoring survival has been carried out at national and international level to define the **level of the contamination** and the **toxicological risk**.
- * When found, the contaminated eggs have been withdrawn and destroyed and the farms has been seized till verification analysis.



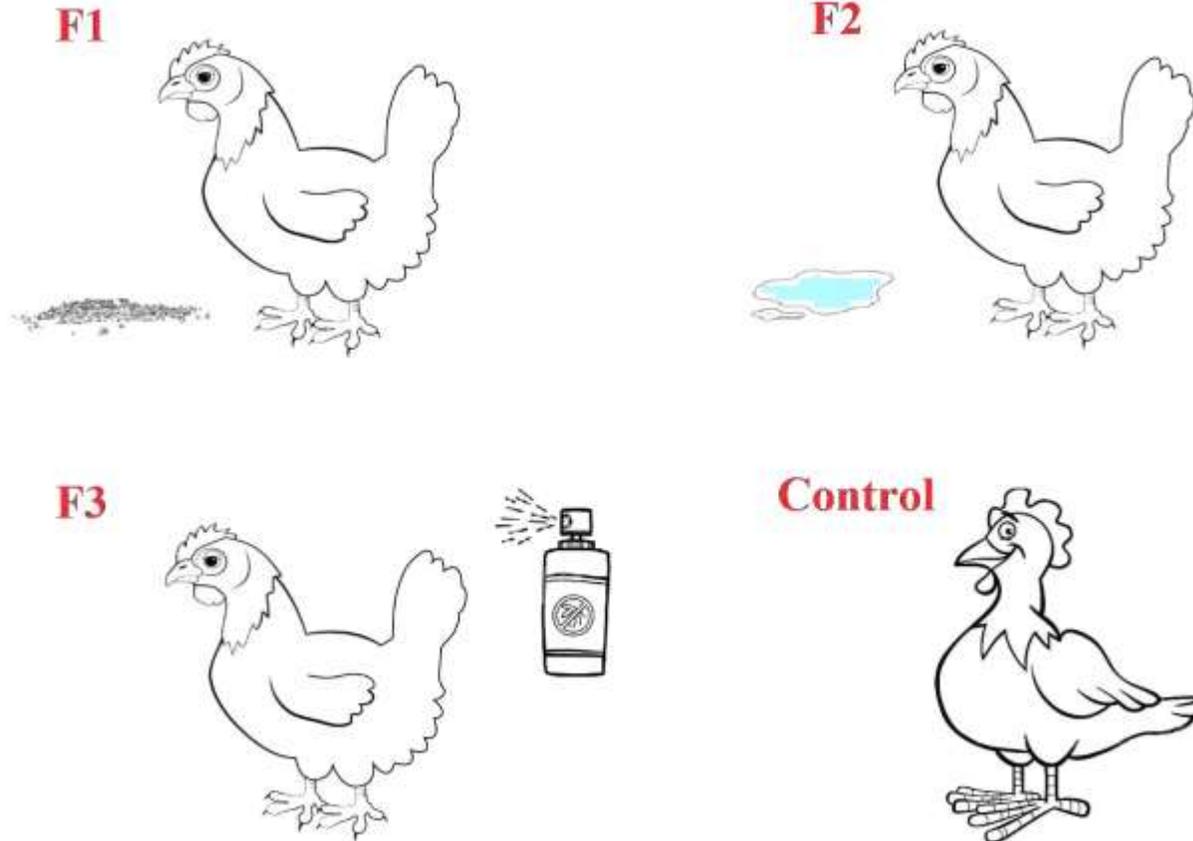
The research aim



- * The intent of this work was to evaluate how different introduction pathways can influence chicken contamination and the level of eggs pollution.

Methodology

4 groups of 5 animals have been selected for the experiment. Each group was subjected to a different treatment:



Treatments with fipronil were made **once a week**.

Prevalent contamination path

* More in-depth studies are needed to evaluate the prevalent contamination path, and ongoing experiments will allow to have more information on the distribution of the pesticide in the chicken organism and to select less invasive methods for chicken analysis.

Sample	day(s)	C		F1	
		Fipronil	Fipronil Sulfone	Fipronil	Fipronil Sulfone
		mg/kg ± RSD%		mg/kg ± RSD%	
MRM		435 > 330	451 > 415	435 > 330	451 > 415
Eggs	1	tr	tr	tr	0.021 ± 0,004
	2	tr	tr	tr	0.082 ± 0.002
	3	tr	tr	0.181 ± 0.002	15.951 ± 0.001
	4	tr	tr	0.010 ± 0.001	74.030 ± 0.003

* tr < 0.000 mg/Kg

QuEChERS LC/MS-MS method

1  5 g  +  10 ml  1 min

2  + 
 QuEChERS
(No: 5982 - 6650)  1 min  15 min  5 minuti a 4000 rpm

3 6 ml supernatant + 
 QuEChERS
(No: 5982 - 5056)  1 min  15 min  5 minuti a 4000 rpm

Analysis Parameters

UHPLC – MS/MS



Source Parameters

Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	350	350
Gas Flow (l/min)	10	10
Nebulizer (psi)	30	30
Sheath Gas Heater	350	350
Sheath Gas Flow	12	12
Capillary (V)	4000	3000
V Charging	0	0

Fipronil MRM Method

Time Segment 1

Scan Segments

Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Cell Acc (V)	Ret Time (min)	Ret Window	Polarity
Fipronil	No	435	Unit/Enh (6490)	330	Unit/Enh (6490)	150	16	5	13.69	1	Negative
Fipronil	No	435	Unit/Enh (6490)	250	Unit/Enh (6490)	150	28	5	13.69	1	Negative
Fipronil Sulfone	No	451	Unit/Enh (6490)	415	Unit/Enh (6490)	150	17	5	14.24	1	Negative
Fipronil Sulfone	No	451	Unit/Enh (6490)	282	Unit/Enh (6490)	150	29	5	14.24	1	Negative

FINDINGS

The method used, allowed the determination and quantification of fipronil and its major metabolite fipronil sulfone (**Figure 1** and **Figure 2**).

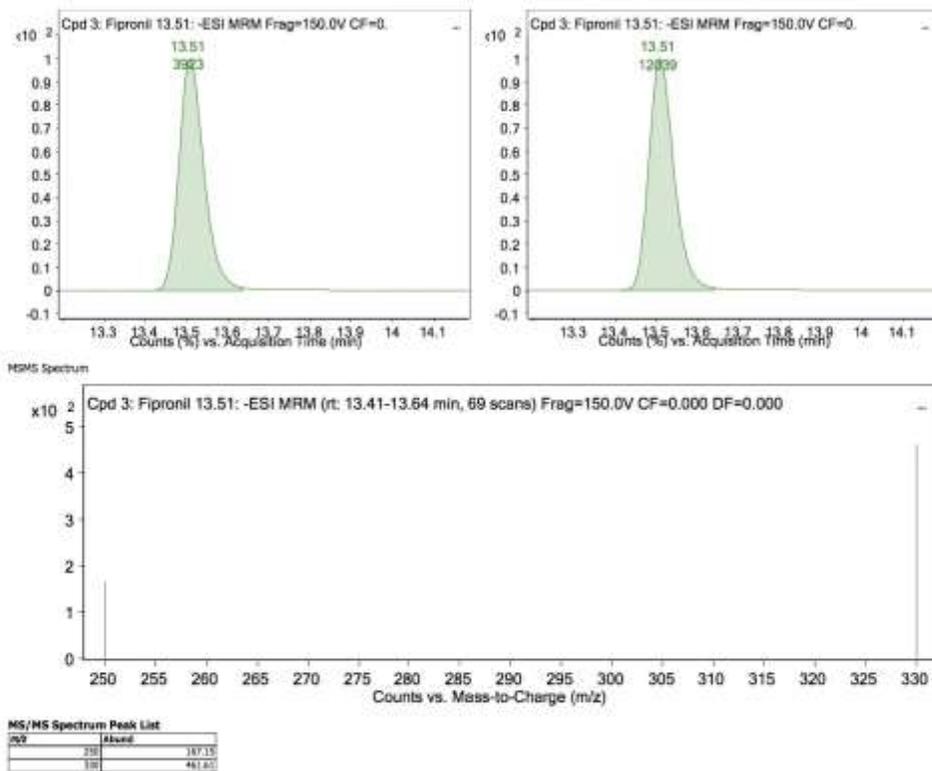


Figure 1. MRM chromatogram and MSMS spectrum of Fipronil

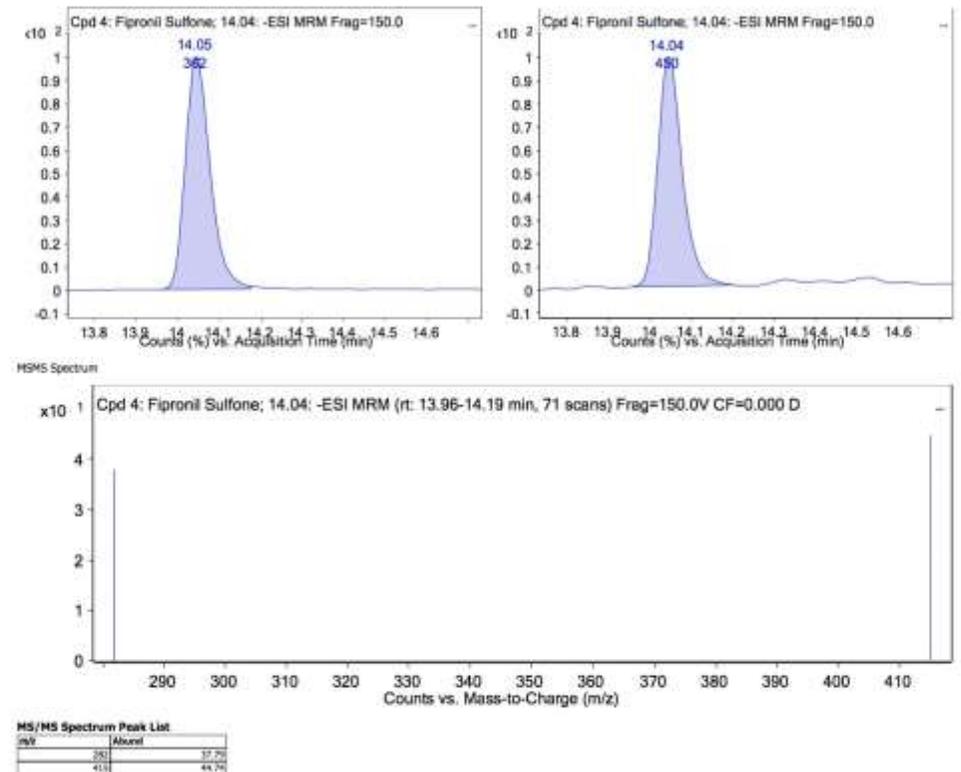


Figure 2. MRM chromatogram and MSMS spectrum Fipronil Sulfone

Conclusion

- * This study carried out on chicken try to elucidate the **behavior of fipronil and fipronil sulfone after oral or parental intake**, aiming to become a reference method for the study of contamination of poultry.
- * More in-depth studies are needed to evaluate the prevalent contamination path, and ongoing experiments will allow to have more information on the distribution of the pesticide in the chicken organism and to **select less invasive methods for chicken analysis.**



THANK YOU FOR YOUR ATTENTION!