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Interdisciplinary Toxicology Program

Short-term atrazine exposure alters the plasma metabolome of male C57BL/6 mice and disrupts specific metabolic pathways

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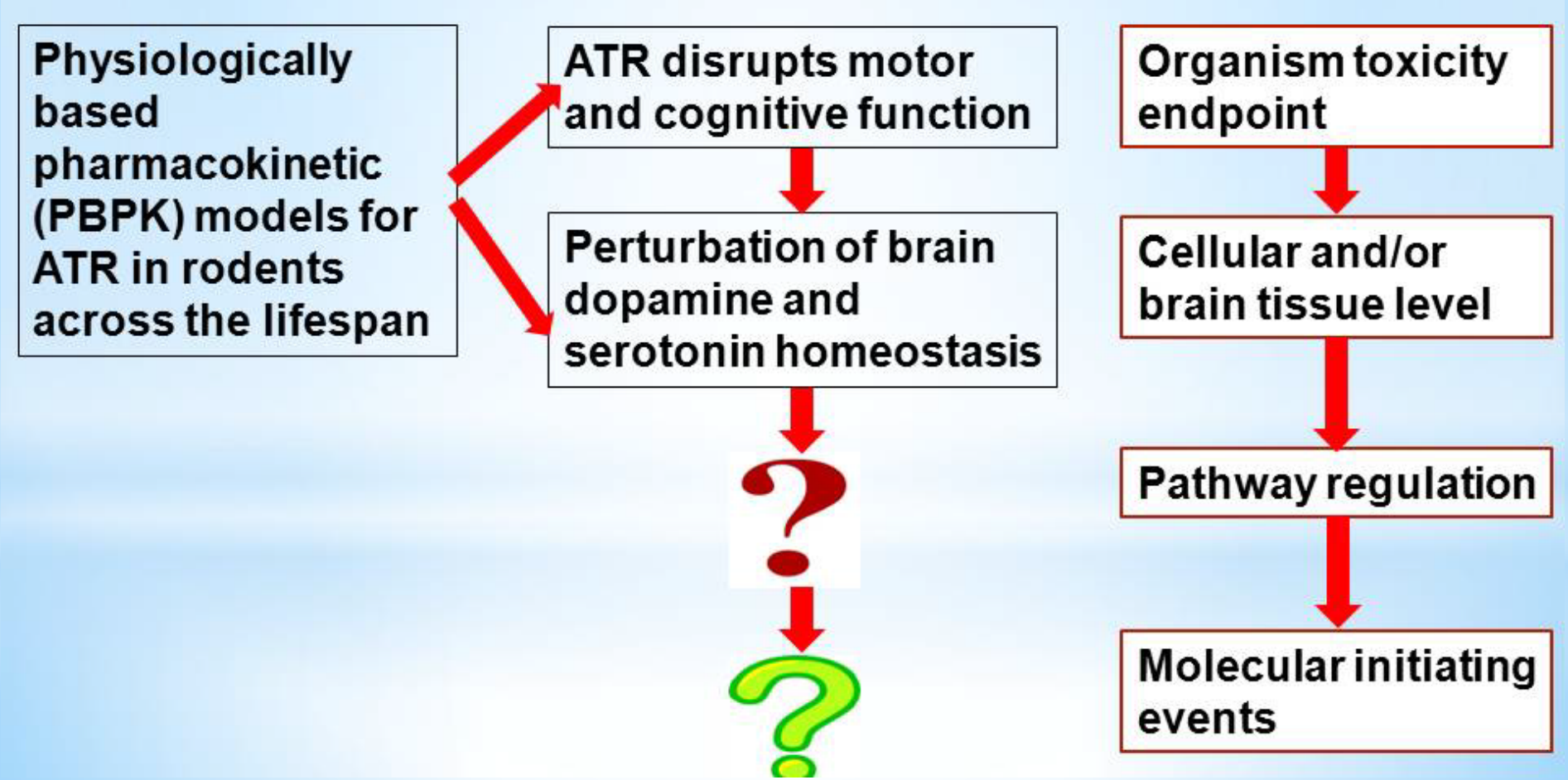
1. Introduction

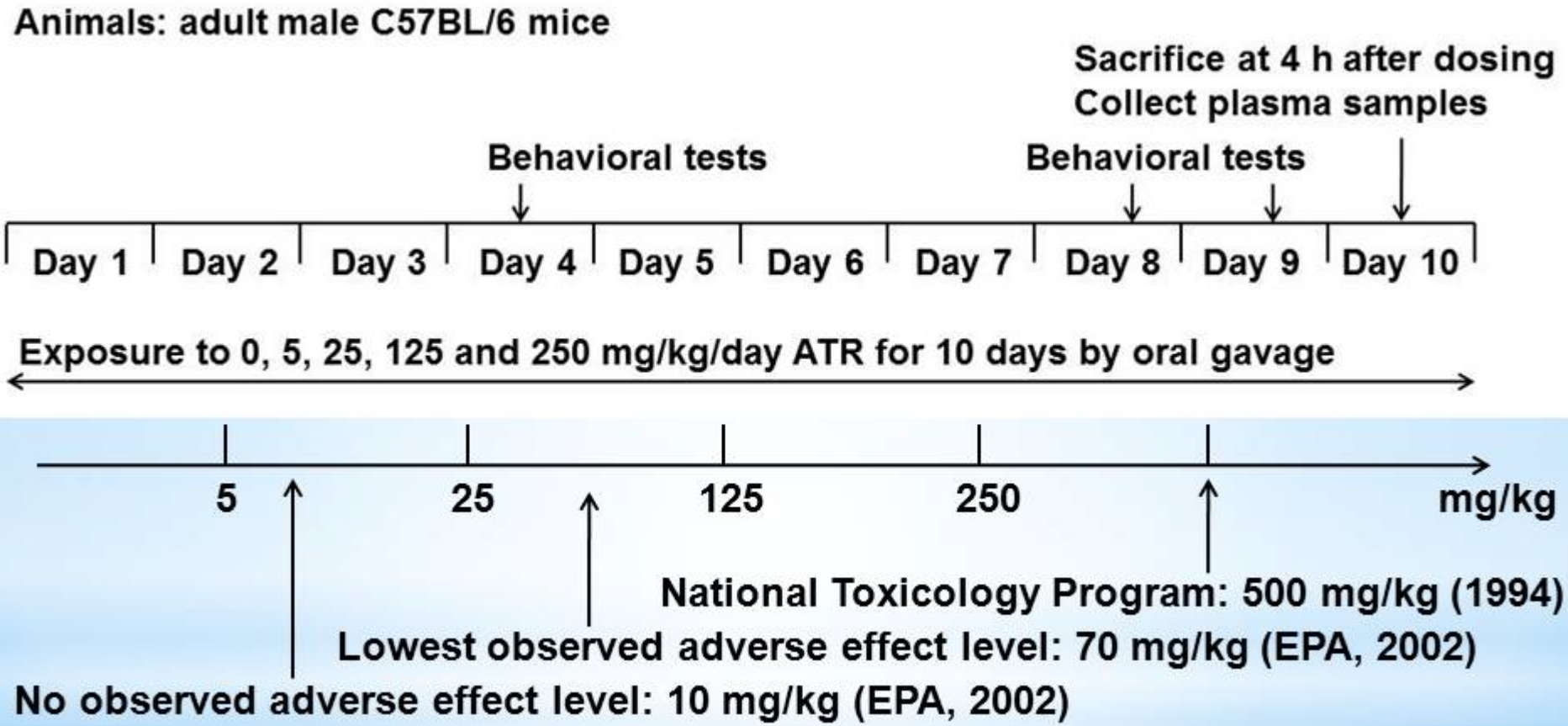
- **Atrazine (ATR) is a widely used herbicide and a ubiquitous environmental contaminant in the US**
- **Possible ATR Adverse Outcome Pathway(s)**



(EPA, 2003; Coban and Filipov, 2007; Lin et al., 2011; Lin et al, 2013a; Lin et al., 2013b; Lin et al., 2013c; Lin et al., 2014; Toccalino et al., 2014)

2. Main methods

- **Experimental design:**

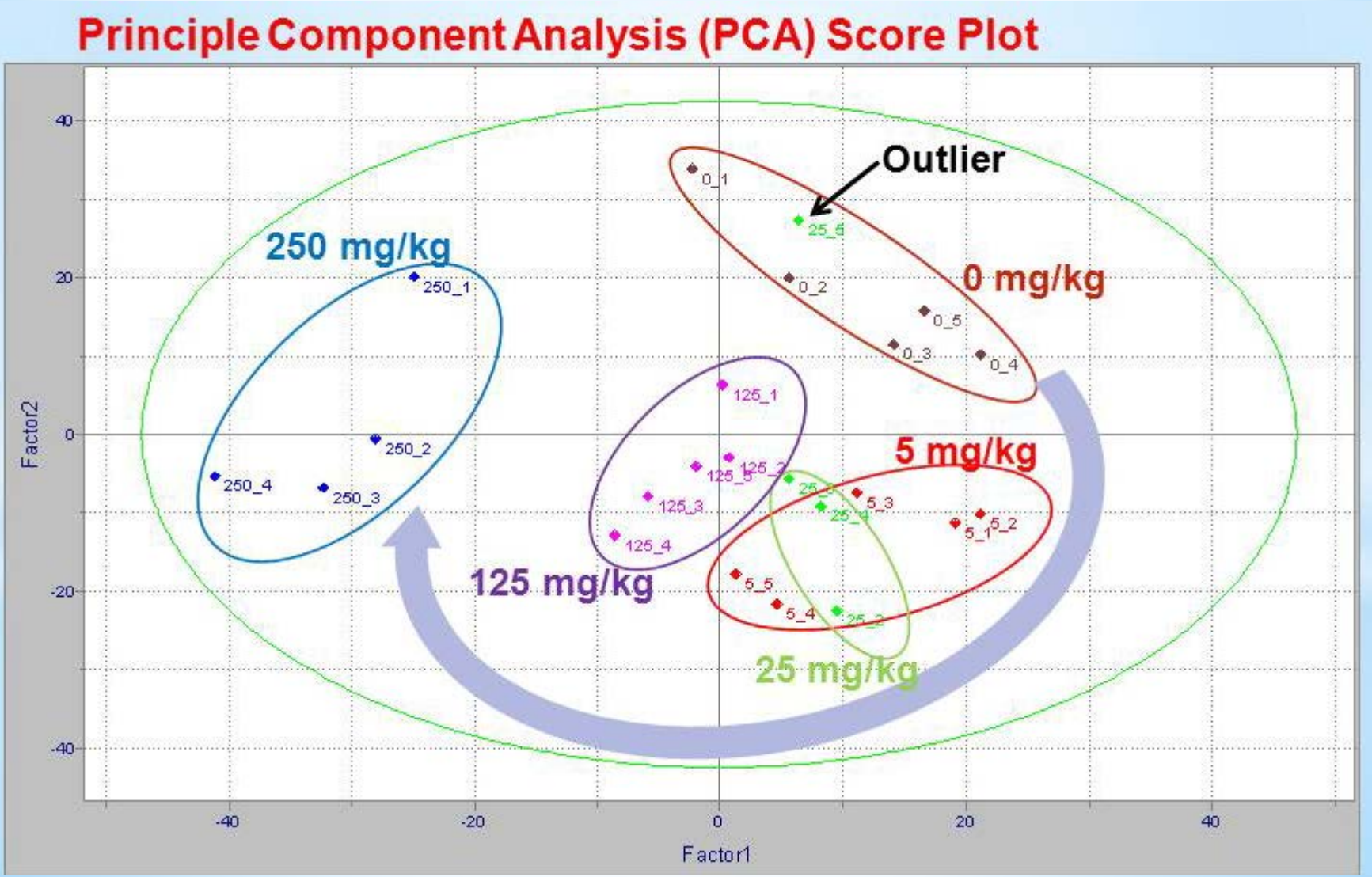


- **Plasma samples were collected on Day 10 and analyzed with high-performance, dual chromatography-Fourier-transform mass spectrometry.**

(Lin et al, 2013a; Soltow et al., 2013)

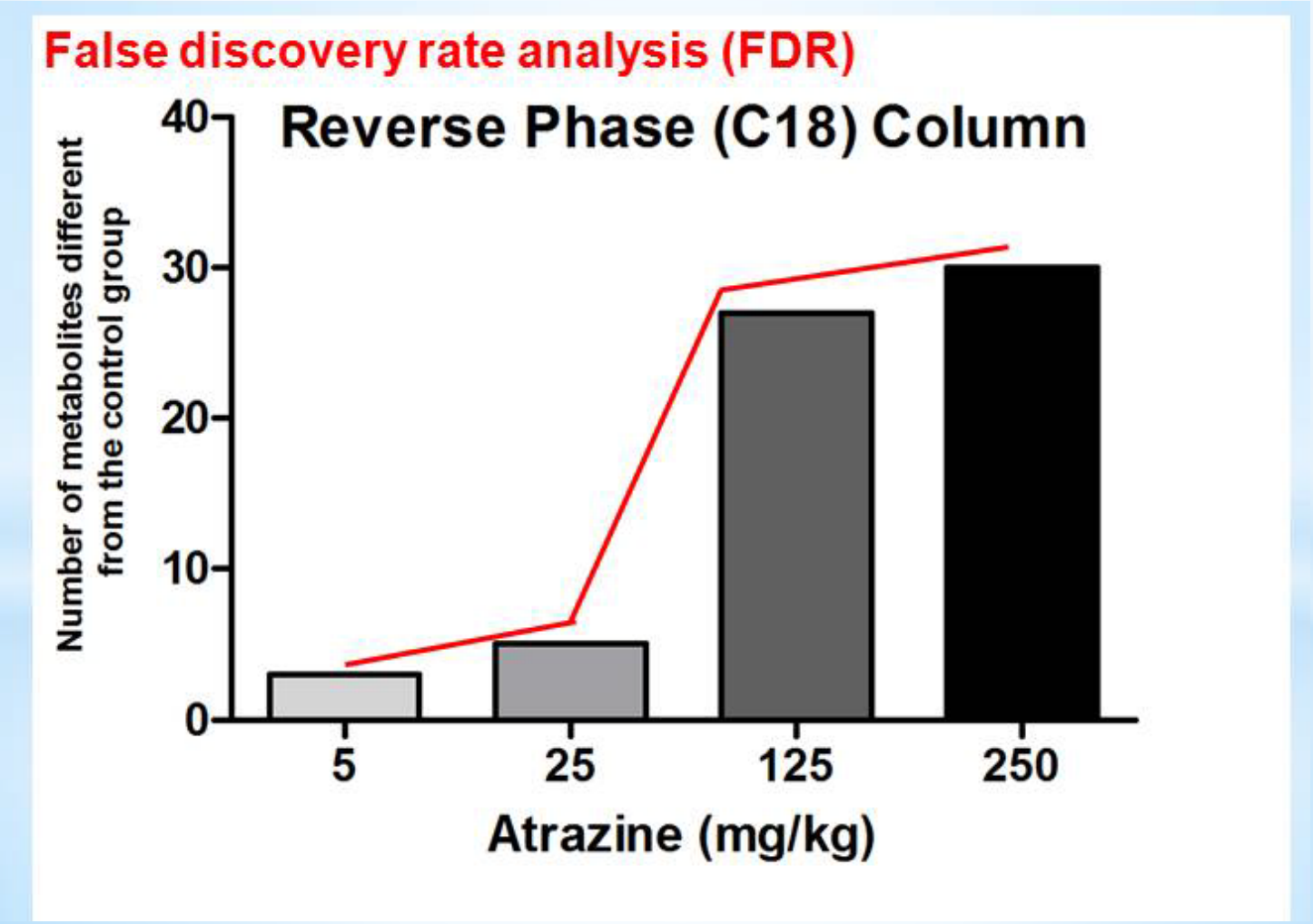
2. Key findings (1)

➢ **ATR exposure resulted in a dose-dependent change of the mouse plasma metabolome.**



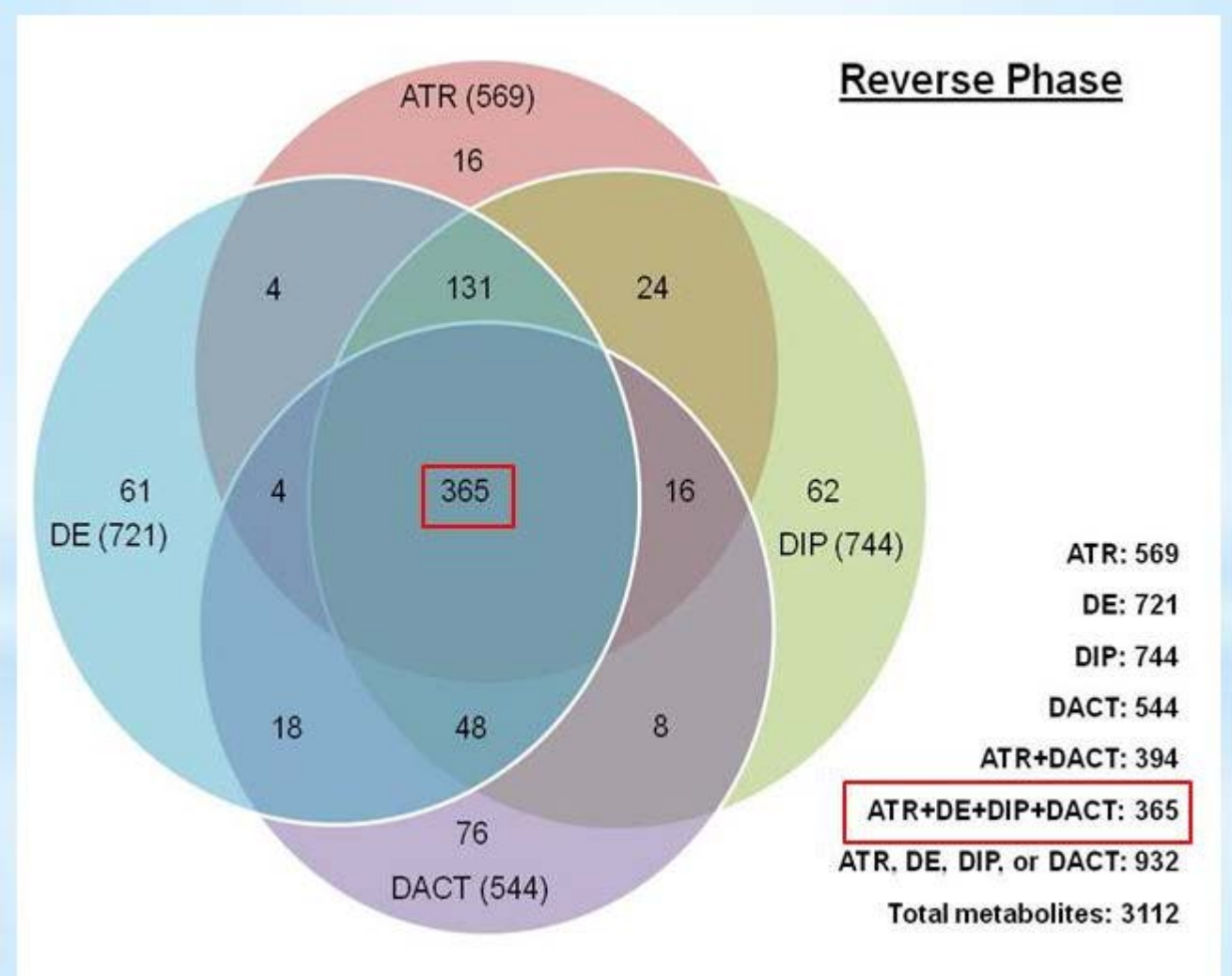
2. Key findings (2)

➢ **ATR exposure dose-dependently increased the number of metabolites with ion intensities significantly different from the control group.**



2. Key findings (3)

- **Pearson correlation analysis showed 365 m/z were strongly correlated with ATR and all its three major chlorinated metabolites.**



2. Key findings (4)

- **Metlin database analysis showed that ATR and its metabolites are strongly correlated with metabolites involving tyrosine, tryptophan, linoleic acid, and α-linolenic acid pathways.**

Table 1. Metabolites of interest that are strongly correlated with atrazine exposure.

m/z	Retention time (s)	Metabolite name	Formula	Adduct	ppm	Correlation coefficient	Correlated atrazine/metabolites
182.081	104.878	**Tyrosine**	C9H11NO3	M+H	0	-0.60	ATR+DE+DIP+DACT
132.102	192.841	Leucine/Isoleucine	C6H13NO2	M+H	3	-0.56	ATR+DE+DIP+DACT
546.355	518.894	LysoPC(20:3)	C28H52NO7P	M+H	1	-0.52	ATR+DE+DIP+DACT
572.367	529.039	LysoPC(22:4)	C30H54NO7P	M+H	7	-0.51	ATR+DE+DIP+DACT
162.112	102.706	Carnitine	C7H15NO3	M+H	1	-0.50	ATR+DE+DIP+DACT
260.187	521.723	Hexanoylcarnitine	C13H25NO4	M+H	4	-0.44	ATR+DE+DIP+DACT
166.086	100.046	Phenylalanine	C9H11NO2	M+H	1	-0.36	ATR+DE+DIP+DACT
428.376	499.214	Stearoylcarnitine	C25H49NO4	M+H	5	-0.36	ATR+DE+DIP+DACT
190.050	525.208	**Kynurenic acid**	C10H7NO3	M+H	1	0.42	ATR+DE+DIP+DACT
279.233	507.641	**Linolenic Acid**	C18H30O2	M+H	2	0.53	ATR+DE+DIP+DACT
246.075	173.374	Proglinazine	C8H12ClN5O2	M+H	1	0.60	ATR+DE+DIP+DACT
204.064	149.866	**Indolepyruvate**	C11H9NO3	M+H	5	0.67	ATR+DE+DIP+DACT
172.038	122.136	Dihydroxyindole	C8H7NO2	M+Na	6	0.84	ATR+DE+DIP+DACT
205.097	105.479	**Tryptophan**	C11H12N2O2	M+H	1	-0.57	ATR+DACT
450.358	468.727	Stearoylcarnitine	C25H49NO4	M+Na	5	0.31	ATR+DACT

2. Key findings (5)

➢ **ATR exposure altered ion intensities of metabolites involving tyrosine, tryptophan, linoleic acid, and α-linolenic acid pathways.**

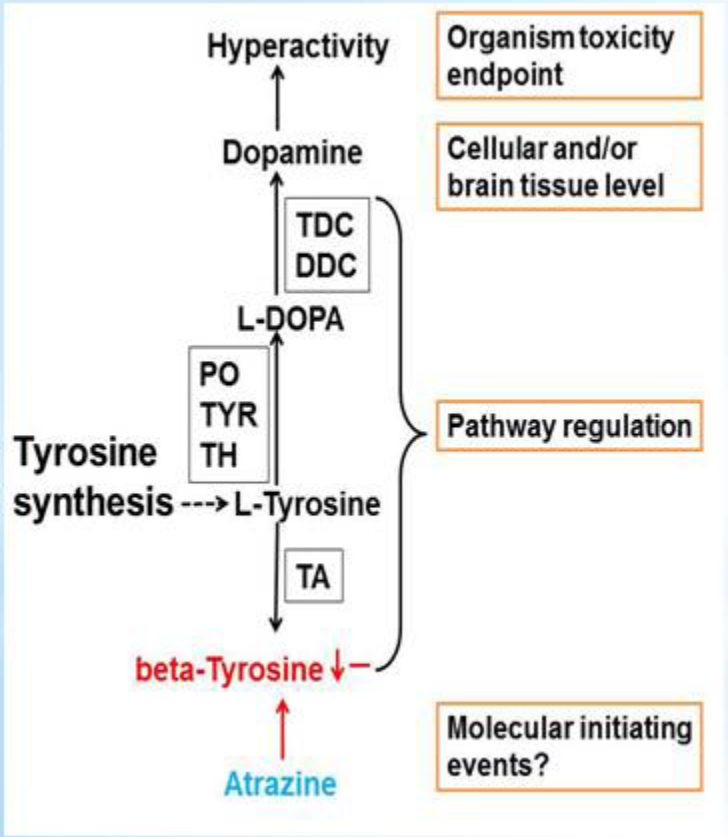
Table 2. Ion intensities of metabolites of interest in control and atrazine-treated (125 mg/kg) groups.

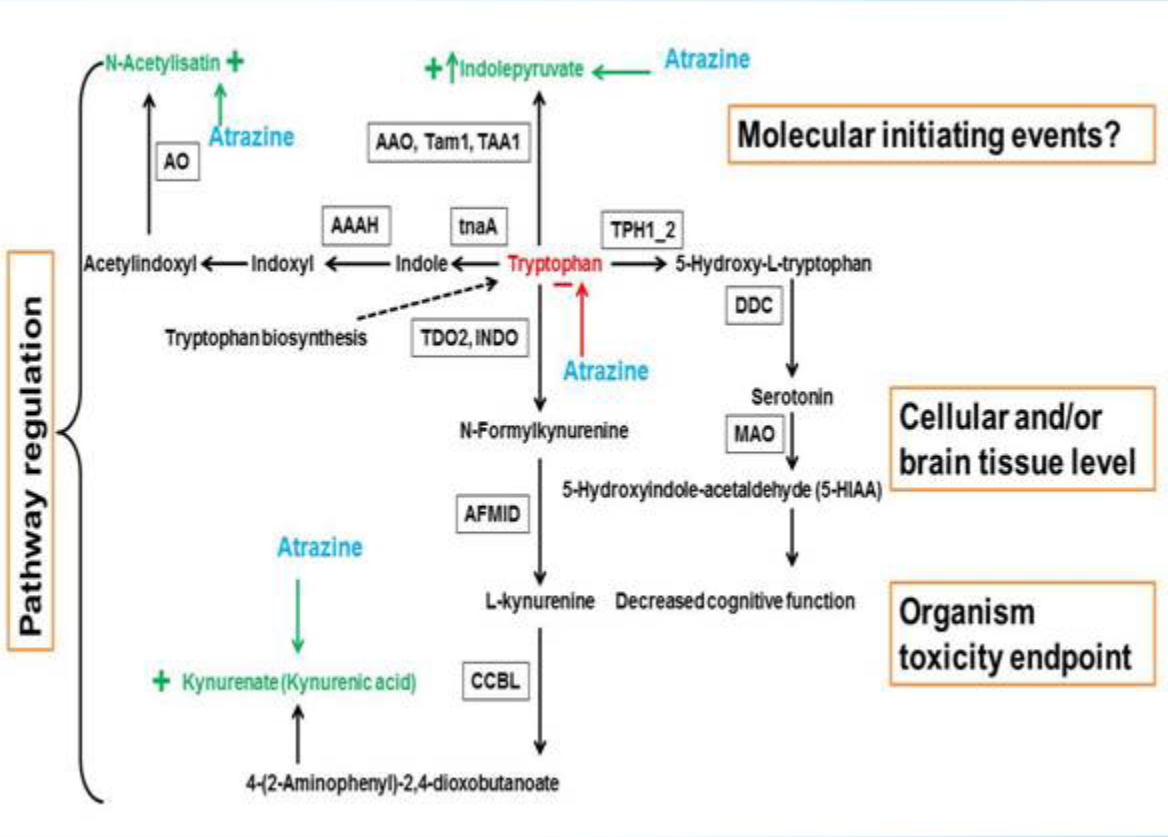
Pathway/Metabolite	m/z ratio	Adduct	Control	125 mg/kg atrazine	p value
Tyrosine metabolism					
Beta-tyrosine	182.0810	M+H	189283.992 ± 37488.177	98210.812 ± 27956.879	0.087
Tryptophan metabolism					
Tryptophan	205.0969	M+H	1019420.962 ± 147828.090	645464.129 ± 213771.769	0.188
Indolepyruvate	204.0643	M+H	1947.036 ± 168.859	66304.072 ± 28804.879	0.095
Kynurenic acid	190.0501	M+H	31028.104 ± 16880.597	23194.91 ± 2871.946	0.660
N-Acetylisatin	190.0501	M+H	31028.104 ± 16880.597	23194.91 ± 2871.946	0.660
Indolepyruvate/Tryptophan ratio	-	M+H	0.00168 ± 0.000133	0.180 ± 0.0907	0.095
Kynurenic acid/Tryptophan ratio	-	M+H	0.0338 ± 0.0223	0.0717 ± 0.0282	0.322
N-Acetylisatin/Tryptophan ratio	-	M+H	0.0338 ± 0.0223	0.0717 ± 0.0282	0.322
Linoleic acid metabolism					
Y-Linolenate	279.2325	M+H	26377.484 ± 10676.857	99976.905 ± 29811.562	0.049
Crepenynate	279.2325	M+H	26377.484 ± 10676.857	99976.905 ± 29811.562	0.049
9-OxoODE	295.2274	M+H	174688.392 ± 46316.891	227818.573 ± 42553.150	0.423
13-OxoODE	295.2274	M+H	174688.392 ± 46316.891	227818.573 ± 42553.150	0.423
α-Linolenic acid metabolism					
α-Linolenic acid	279.2325	M+H	26377.484 ± 10676.857	99976.905 ± 29811.562	0.049
13(S)-HpOTrE	311.2223	M+H	84042.802 ± 22155.600	325147.659 ± 66906.121	0.009
12,13EOTrE	293.2113	M+H	63114.041 ± 21272.621	143606.124 ± 19669.706	0.024
12-OPDA	293.2113	M+H	63114.041 ± 21272.621	143606.124 ± 19669.706	0.024
OPC8	295.2274	M+H	174688.392 ± 46316.891	227818.573 ± 42553.150	0.423
13(S)-HpOTrE/α-Linolenic acid	-	M+H	19.384 ± 11.852	4.658 ± 1.519	0.253
12,13-EOTrE/α-Linolenic acid	-	M+H	6.821 ± 2.759	2.142 ± 0.706	0.139
12-OPDA/α-Linolenic acid	-	M+H	6.821 ± 2.759	2.142 ± 0.706	0.139
OPC8/α-Linolenic acid	-	M+H	73.522 ± 42.879	3.742 ± 1.951	0.143

2. Key findings (6)

➢ **Proposed ATR Adverse Outcome Pathways (AOPs).**

A. Hyperactivity AOP B. Cognitive deficit AOP





3. Summary

- **ATR alters plasma metabolome and disrupts multiple metabolic pathways.**
- **ATR-induced perturbation of periphery tyrosine and tryptophan metabolism may be reflective of the previously reported alterations of brain dopamine and serotonin homeostasis.**
- **Two AOPs for ATR toxicity are proposed. However, additional studies are needed to verify these results and to identify molecular initiating events involved in these AOPs.**
- **The alterations in the plasma metabolome, especially of the α-linolenic acid and linoleic acid metabolic pathways, are potential novel and sensitive biomarkers of ATR toxicity, and could be used for identification of novel AOPs.**

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