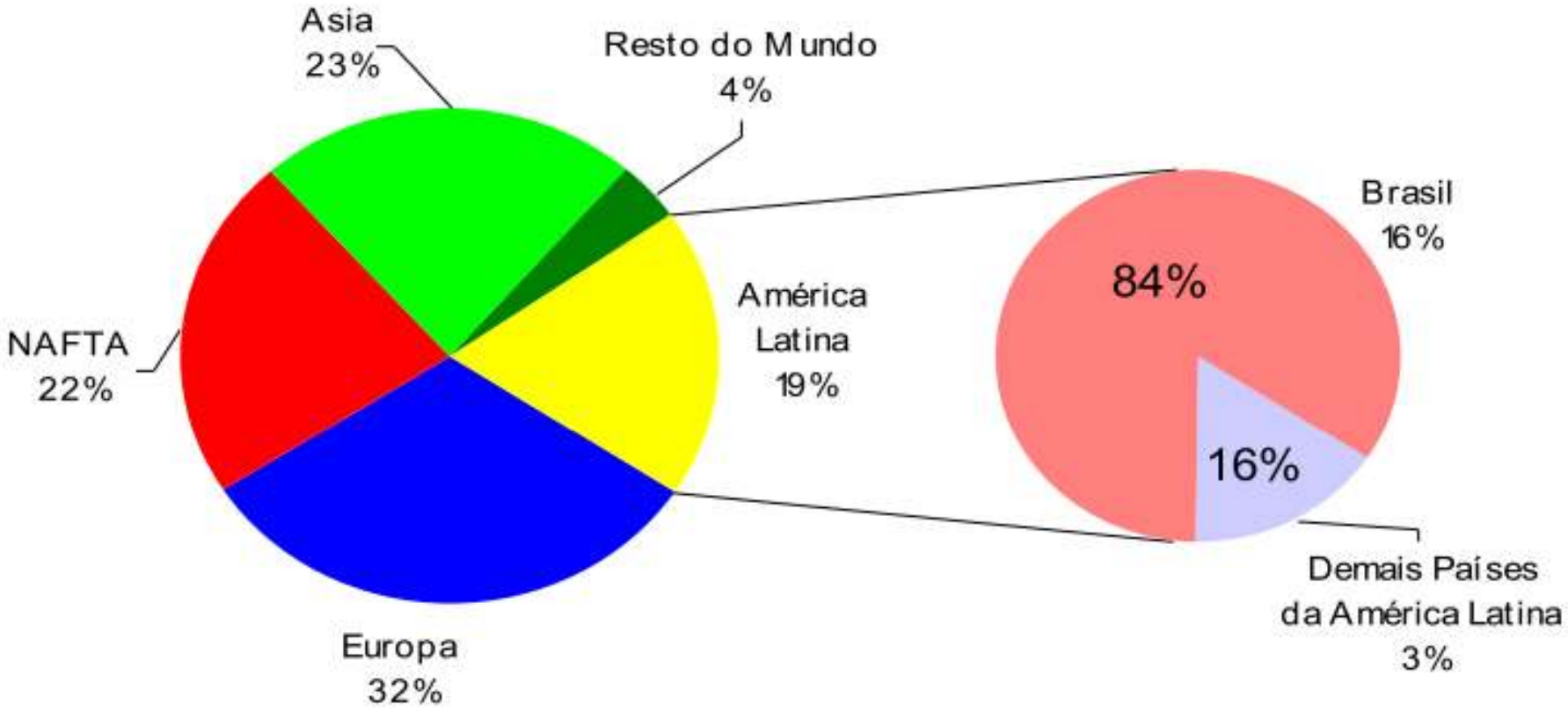
Biomarcadores para a avaliação e monitoramento dos recursos hídricos em áreas rurais

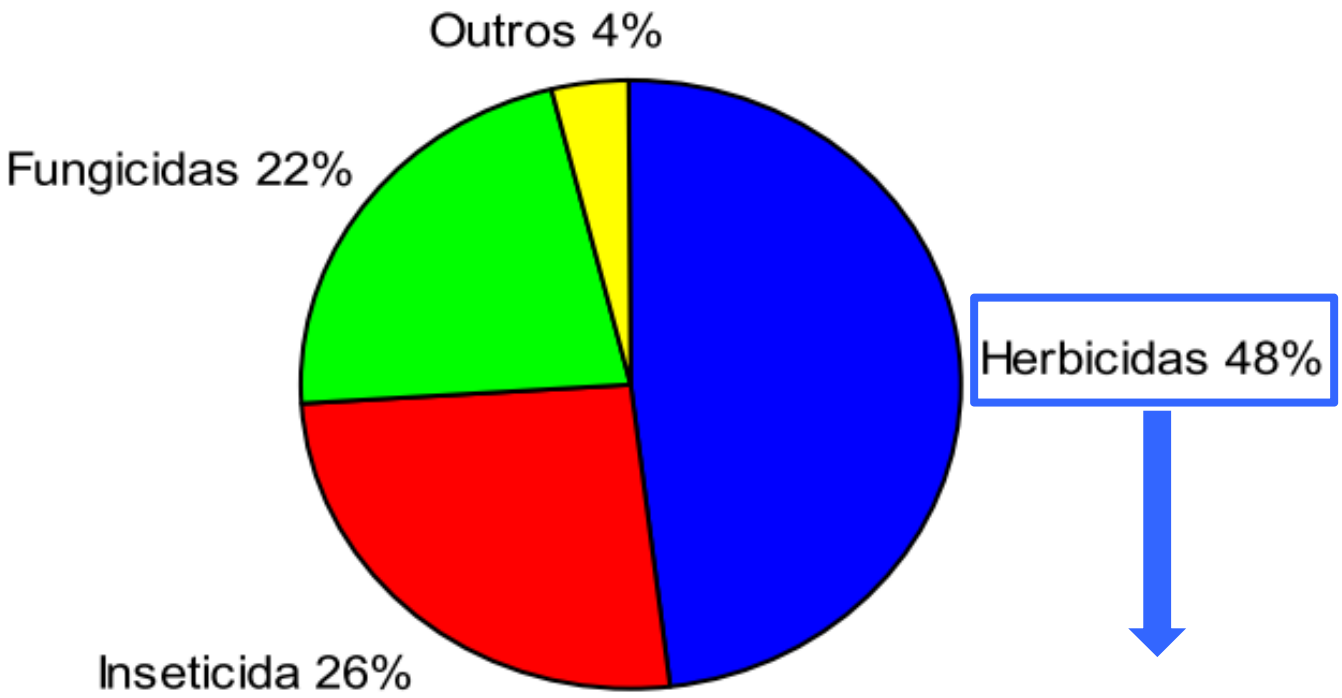
Cláudia Bueno dos Reis Martinez
Laboratório de Ecofisiologia Animal
Universidade Estadual de Londrina

Venda Mundial de Agrotóxicos em 2007



BRASIL ➡ **4º lugar no ranking mundial**
80% do consumo da América Latina

Consumo Mundial Médio de Agrotóxico por Classe de Uso (de 2000 a 2007)



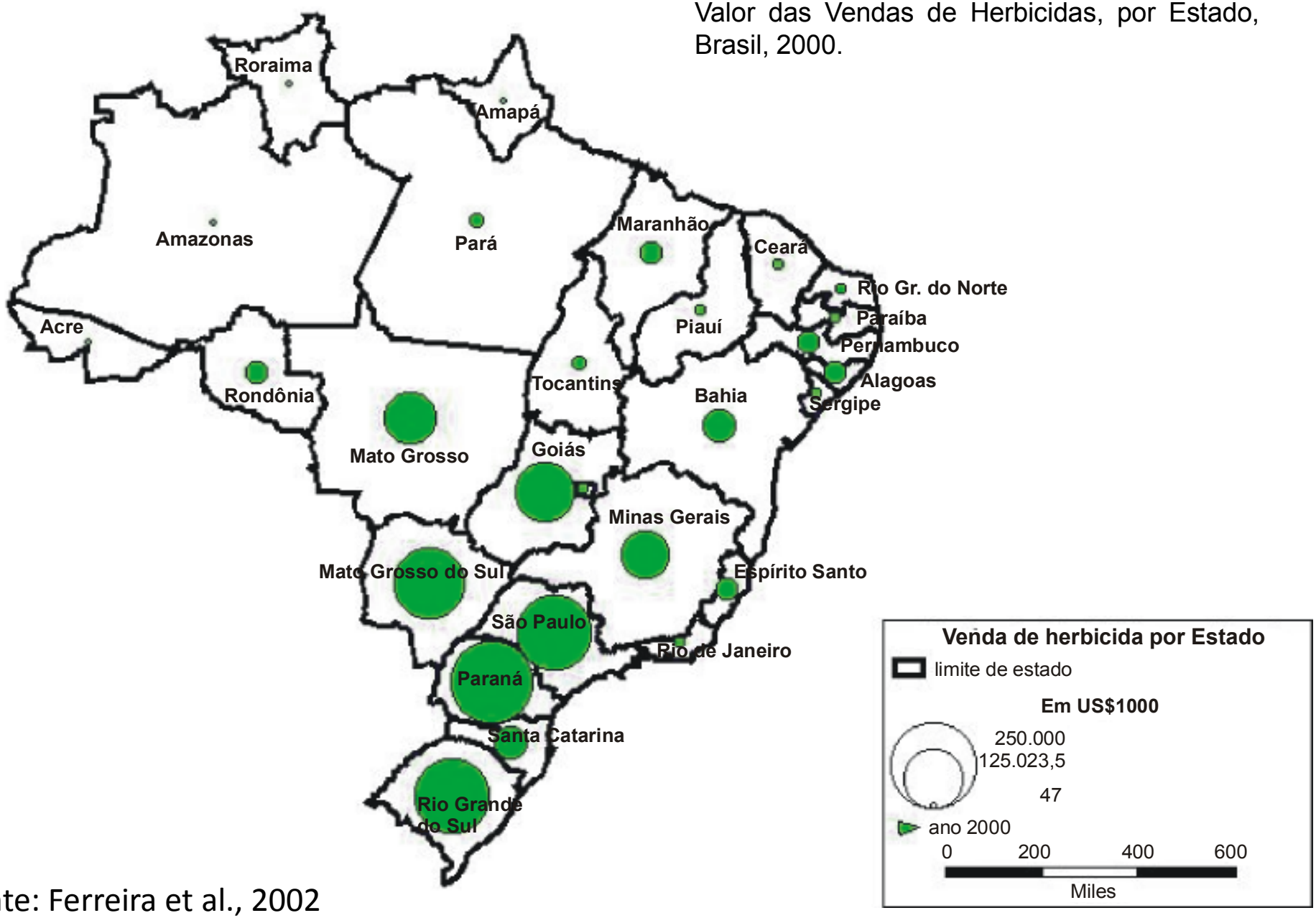
NO BRASIL:

✓em 2008 o consumo de herbicidas correspondeu a mais de 59% do total de agrotóxicos

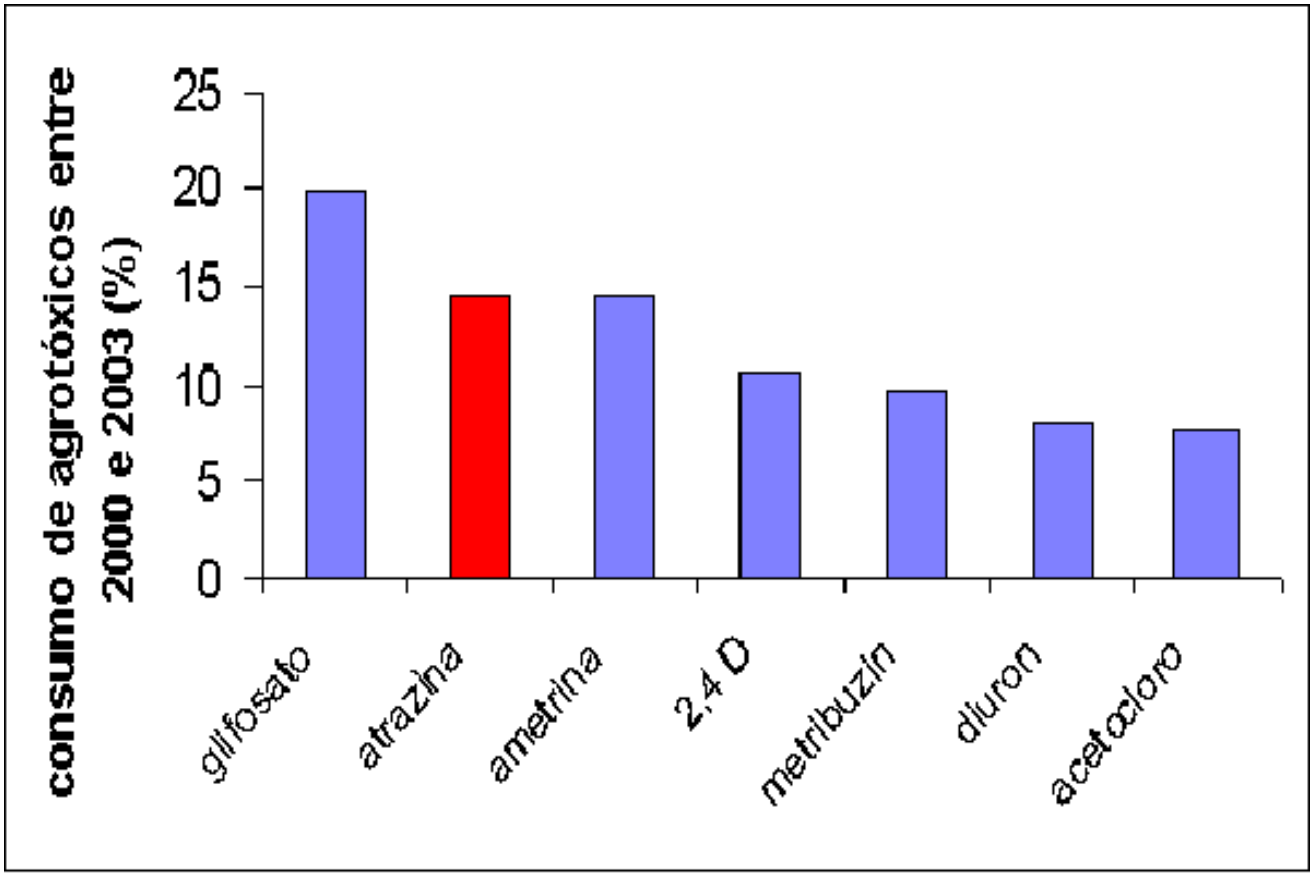
✓principalmente nas culturas da soja, milho e cana-de-açúcar

DISTRIBUIÇÃO TERRITORIAL DAS VENDAS DE HERBICIDAS NO BRASIL

Valor das Vendas de Herbicidas, por Estado, Brasil, 2000.



Fonte: Ferreira et al., 2002



(ARMAS, 2005)

Distribuição, no período de 4 anos (2000 a 2003), do consumo de agrotóxicos na sub-bacia do rio Corumbataí (SP). Armas et al, 2005.

Volume de comercialização de herbicidas, classificados por princípio ativo, mais comercializados no Paraná, no ano de 2000

Nome comum	Total comercializado	
	toneladas de i.a.	% em relação ao total
Glyphosate	4.562,28	29,85
Atrazine	3.075,91	20,12
2,4-D	1.659,33	10,85
Sulfosate	631,60	4,13
Simazine	568,38	3,72
Trifluralin	550,91	3,60
MSMA	436,92	2,58
Metolachlor	387,99	2,54
Diuron	363,23	2,37
Ametryne	300,35	1,96
Paraquat	235,51	1,54
Alachlor	197,87	1,29
Bentazon	125,51	0,82
Imazaquin	119,31	0,78
Acetochlor	98,28	0,64
Cloransulam-metil	88,77	0,58
Fomesafen	86,23	0,57
Clethodim	85,16	0,56
Pendimethalin	84,01	0,55
Outros	1.625,62	10,63

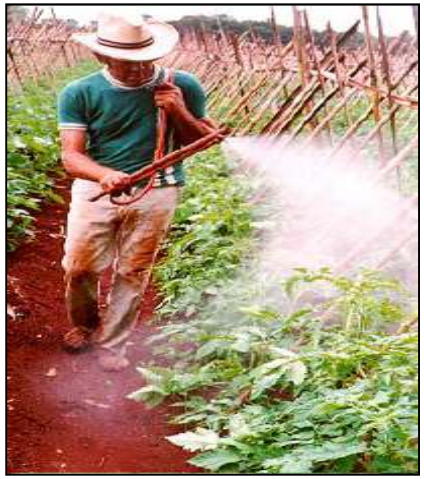
Glifosato

+

Atrazina

50 % do total

Glifosato



Soja, milho, arroz,
café, *Citrus*, maçã,
fumo, uva, pastagens

Culturas RR

Cana-de-açúcar

Aumento do consumo

Roundup Ready
Tecnologia e velocidade com melhores resultados.

Herbicida Roundup®

- Começou a ser produzido no Brasil em 1984;
- Classificado como pouco perigoso ao meio ambiente (Classe IV)
- 48% de glifosato (sal de isopropilamina) + polioxietilenoamino (POEA)

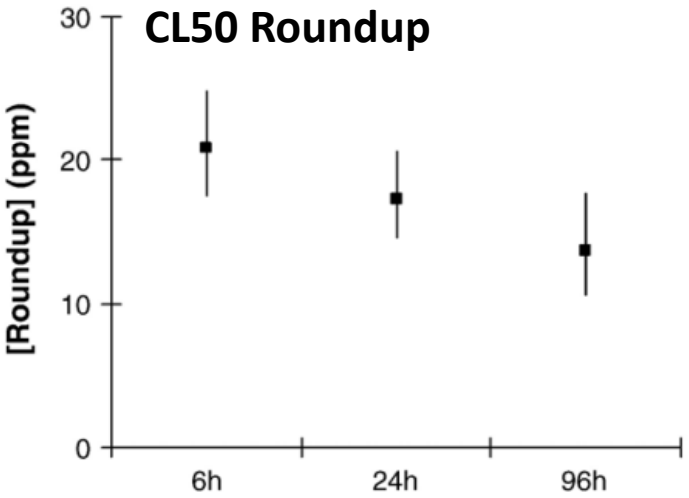
Surfactante facilita a entrada do glifosato

Available online at www.sciencedirect.com
ScienceDirect
Comparative Biochemistry and Physiology, Part C 147 (2008) 222 – 231
CBP
www.elsevier.com/locate/cbpc

Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus*

Vivian do Carmo Langiano, Cláudia B.R. Martinez *

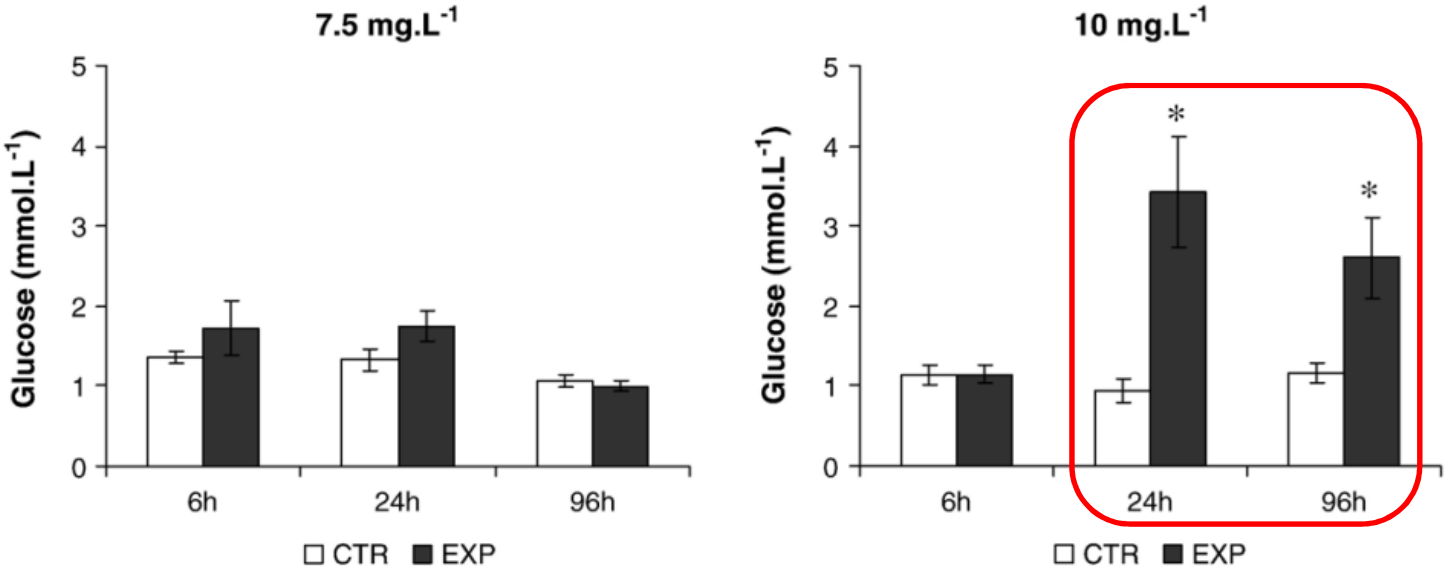
Juvenis de *Prochilodus lineatus* foram expostos ao Roundup® por até 96 h



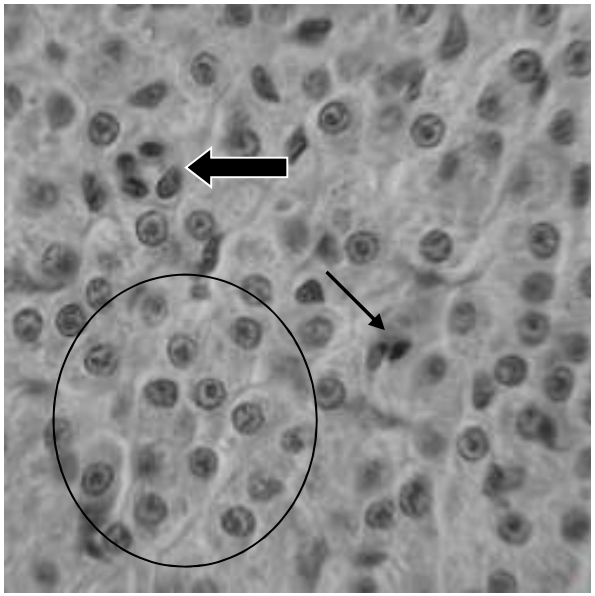
CL_{50} (96h): 13,69 mg L^{-1}

7,5 (55%) e 10 (75%) mg L^{-1}

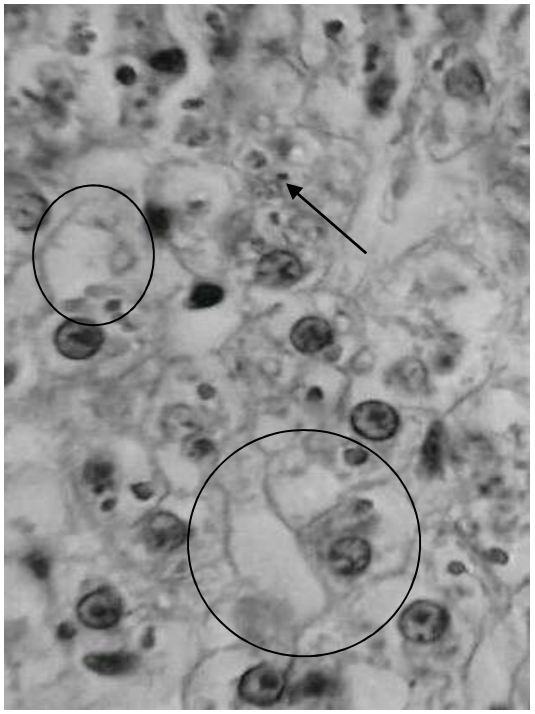
Considerando-se as taxas de aplicação atuais, corpos d´água podem apresentar concentrações de até 3,7 mg L^{-1} glifosato (Giesy et al., 2000), que corresponde a cerca de **9 mg L^{-1} de Roundup®**.



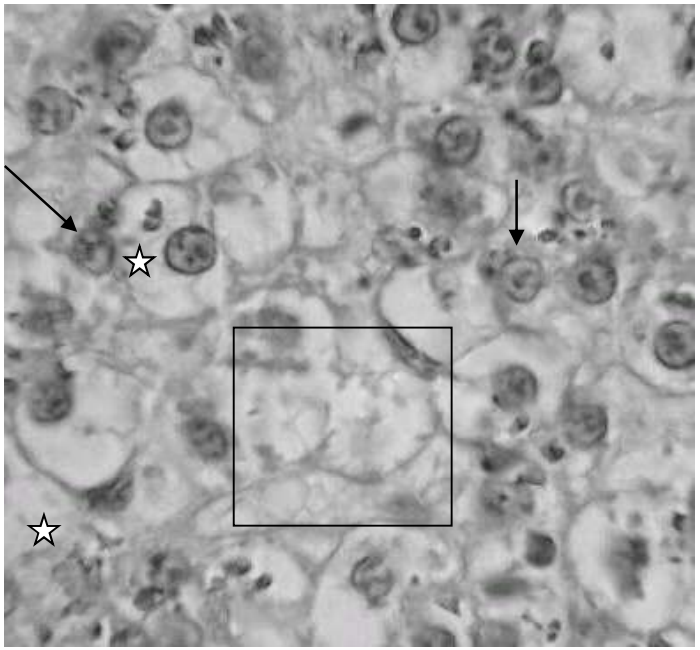
Langiano e Martinez, Comp Biochem Physiol. 147C (2008) . 222-231.



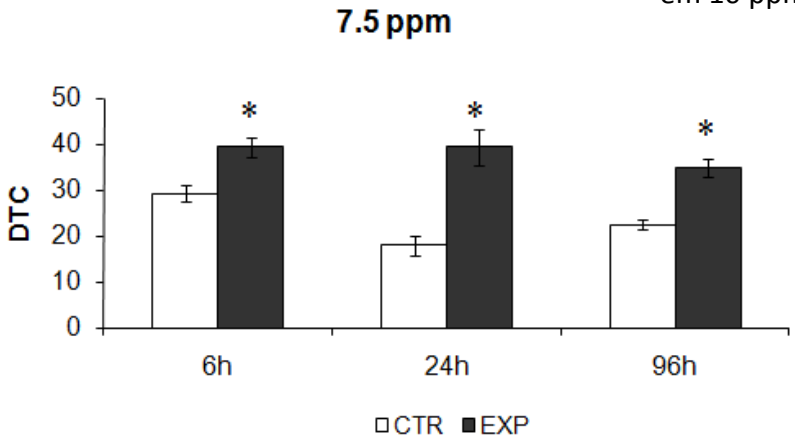
Tecido hepático normal.

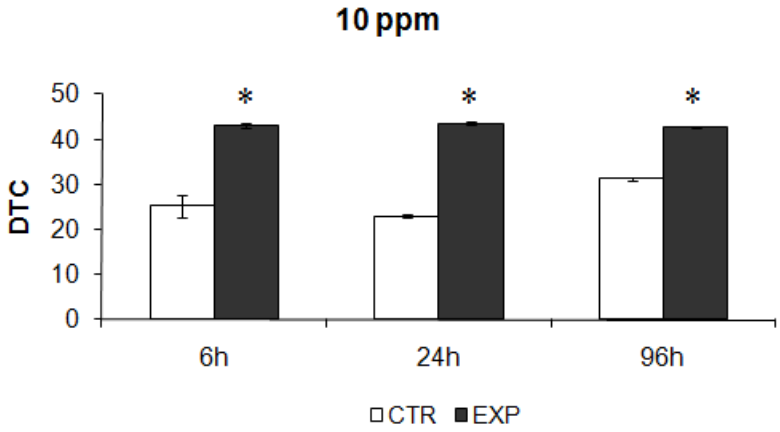


Estagnação biliar (seta) e **vacúolos no citoplasma** (círculos) após 24 h em 10 ppm de Roundup.



Degeneração citoplasmática (quadrado), degeneração nuclear (setas), hipertrofia celular (estrelas) - após 96 h em 10 ppm de Roundup.





Langiano e Martinez, Comp Biochem Physiol. 147C (2008) 222-231.

Chemosphere 78 (2010) 294–299

Contents lists available at ScienceDirect

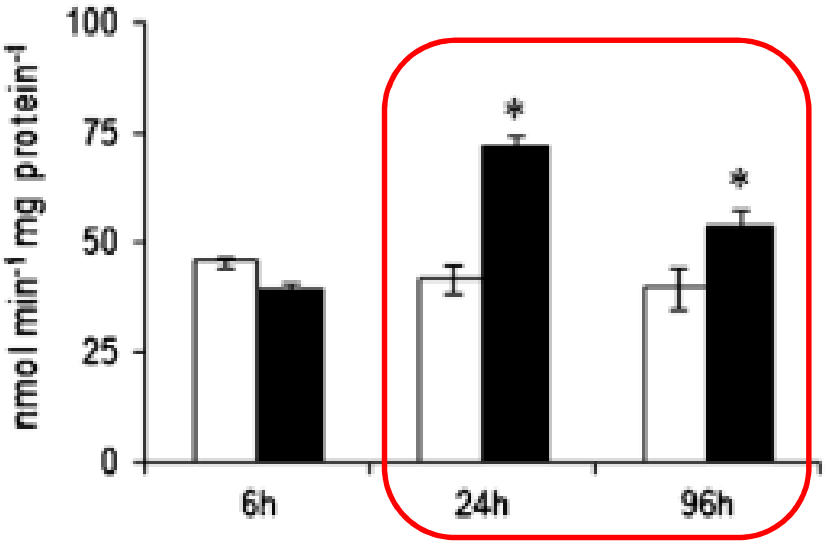
Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Roundup® causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish *Prochilodus lineatus*

Kathya A. Modesto, Cláudia B.R. Martinez *

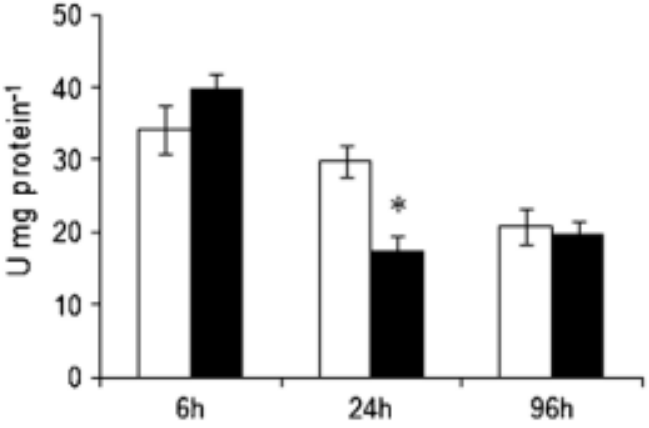
↑ GST



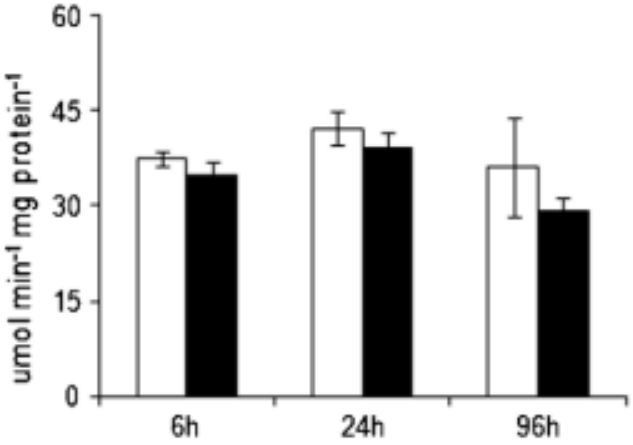
Controle

Roundup (10 mg.L^{-1})

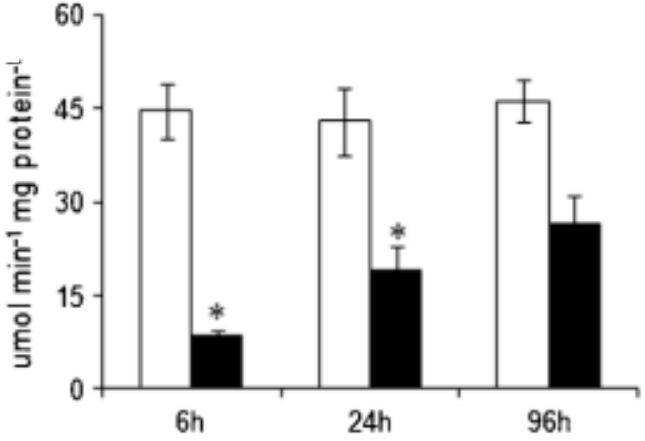
SOD



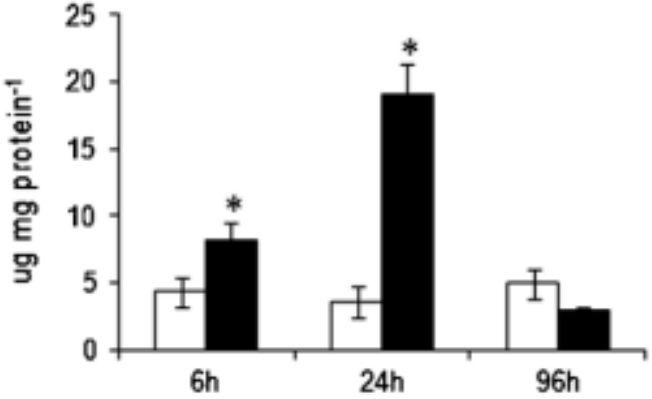
GPx



CAT



GSH

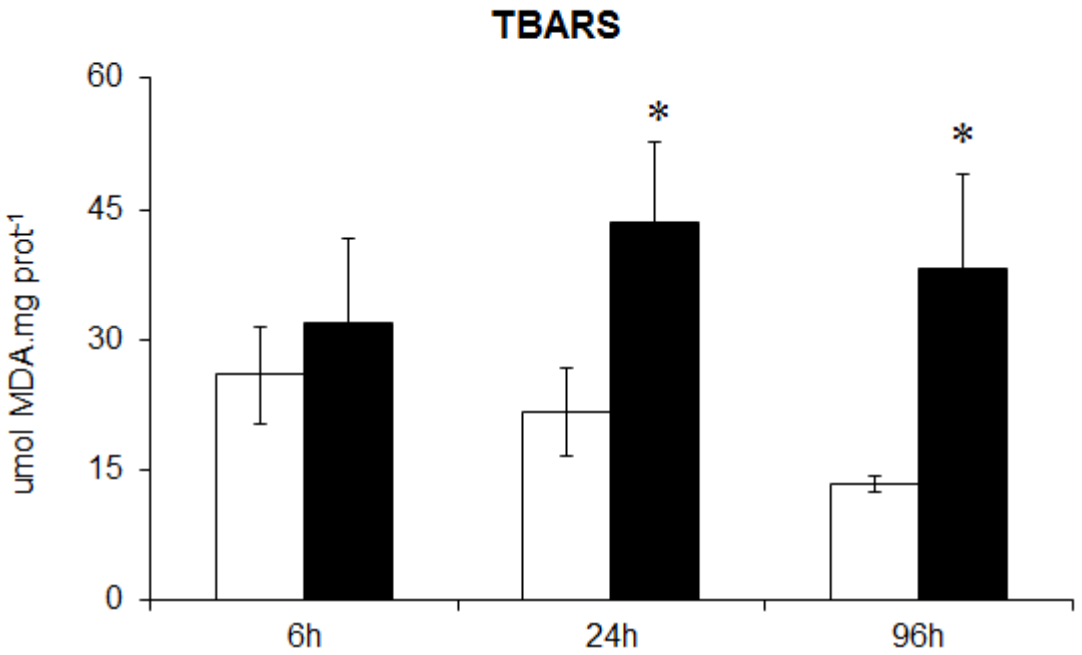


Controle

Roundup (10 mg.L^{-1})

Modesto e Martinez.
Chemosphere 78 (2010) 294-299

Peroxidação lipídica após 24 e 96 h de exposição

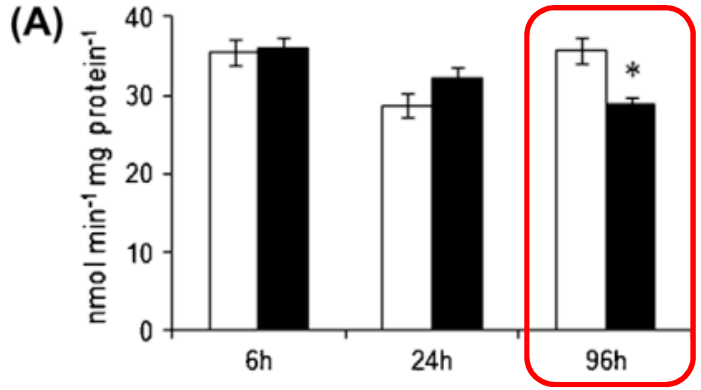


Controle

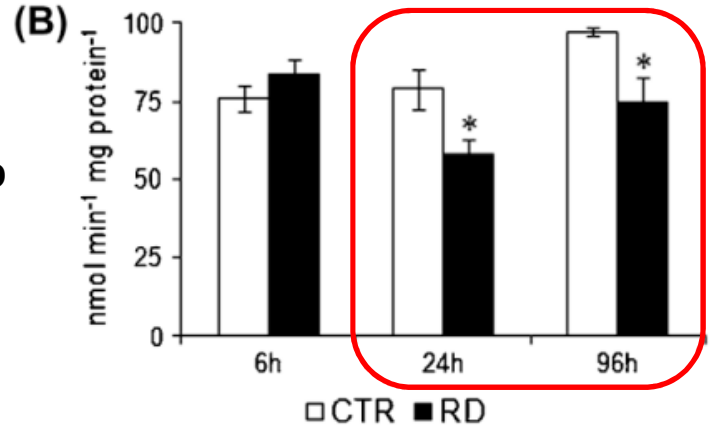
Roundup (10 mg.L^{-1})

Modesto e Martinez.
Chemosphere 78 (2010) 294-299

AChE no cérebro



AChE no músculo



Controle

Roundup (10 mg.L^{-1})

Roundup: promove inibição da acetilcolinesterase no cérebro e músculo de *Prochilodus lineatus*.

Modesto e Martinez.
Chemosphere 78 (2010) 294-299

Mutation Research 655 (2008) 41–46

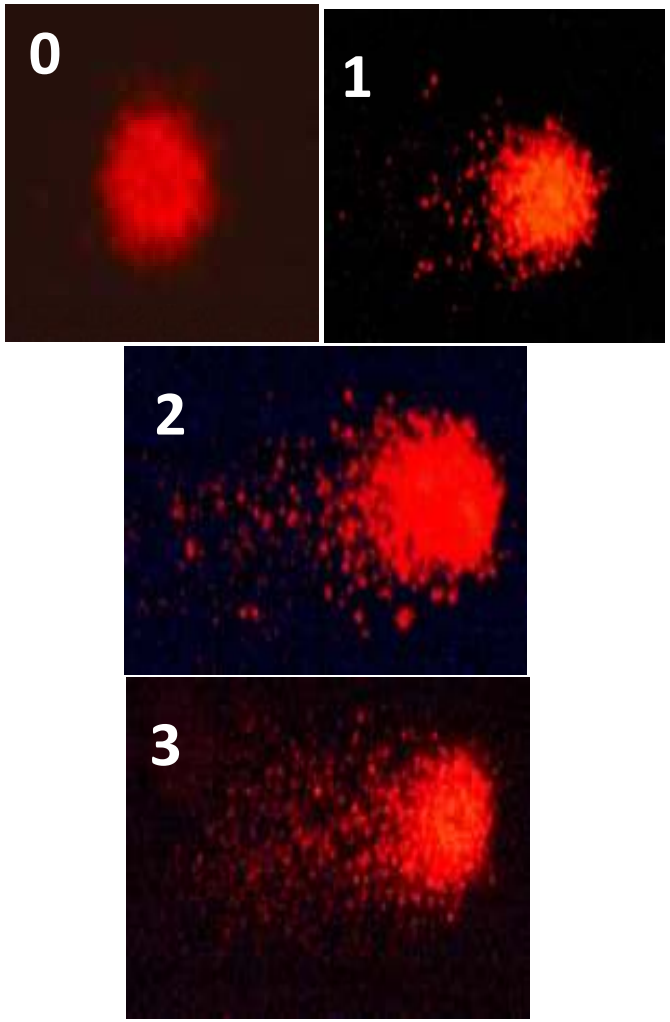
Contents lists available at ScienceDirect

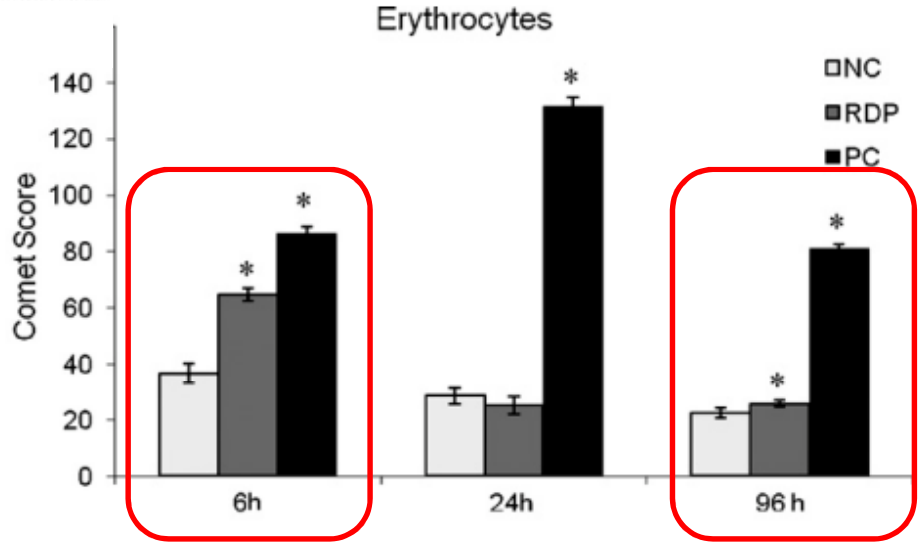
Mutation Research/Genetic Toxicology and Environmental Mutagenesis

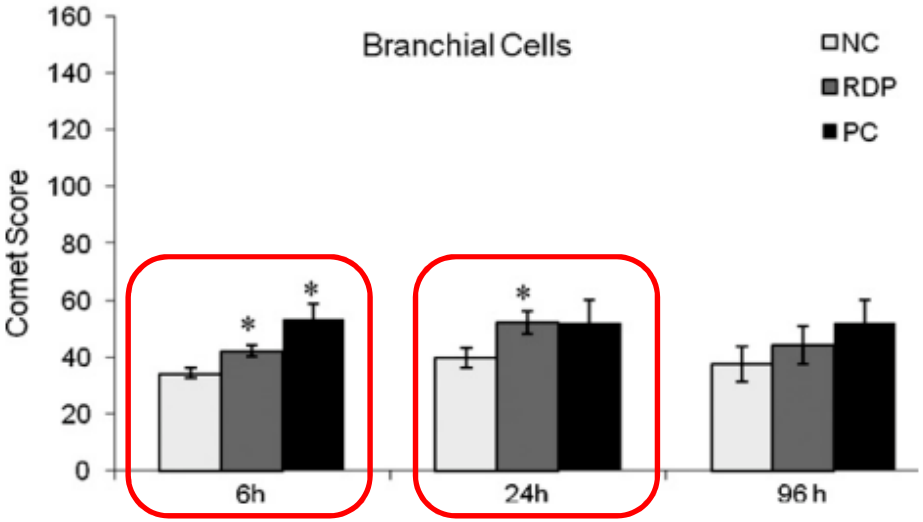
journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

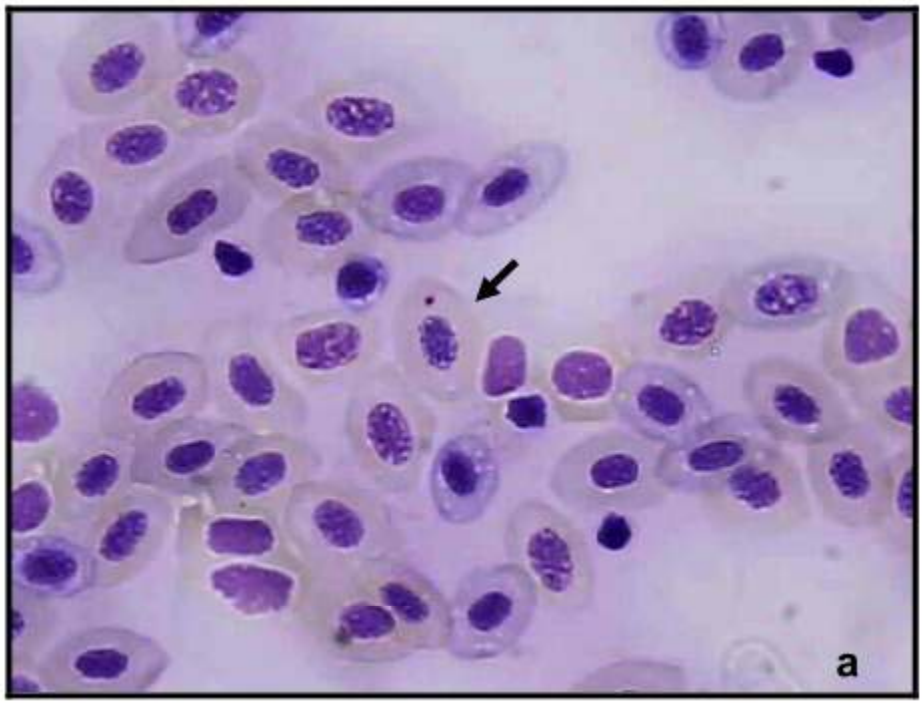
Genotoxic effects of Roundup® on the fish *Prochilodus lineatus*

D.G.S.M. Cavalcante[a], C.B.R. Martinez[b], S.H. Sofia[a,*]









Time	Group	N	MN frequency (‰)	ENA frequency (‰)
6 h	NC	8	0	3.00 ± 0.34
	RDP	10	0	2.37 ± 0.16
	PC	7	0.05 ± 0.05	2.33 ± 0.38
24 h	NC	9	0.07 ± 0.05	2.11 ± 0.30
	RDP	12	0.05 ± 0.05	2.33 ± 0.46
	PC	8	0.71 ± 0.22*	2.33 ± 0.35
96 h	NC	9	0.18 ± 0.11	4.11 ± 0.38
	RDP	12	0.11 ± 0.08	3.67 ± 0.36
	PC	8	0.54 ± 0.09*	4.00 ± 0.30

ROUNDUP ®

- ✓ **promoveu danos genotóxicos nas células de sangue e brânquia**
- ✓ **não promoveu danos mutagênicos nos eritrócitos**

Cavalcante et al.
Mutat Res 655 (2008) 41- 46

Roundup Transorb®

- Comercializado desde 1998
- 64,8% glifosato (sal de isopropilamina) + mistura de surfactantes (15% POEA)
- Classificado como perigoso ao meio ambiente (classe III)
- Tecnologia Transorb: translocação mais rápida do produto e em maiores quantidades nas plantas invasoras.

Chemosphere xxx (2010) xxx–xxx

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journal homepage: www.elsevier.com/locate/chemosphere

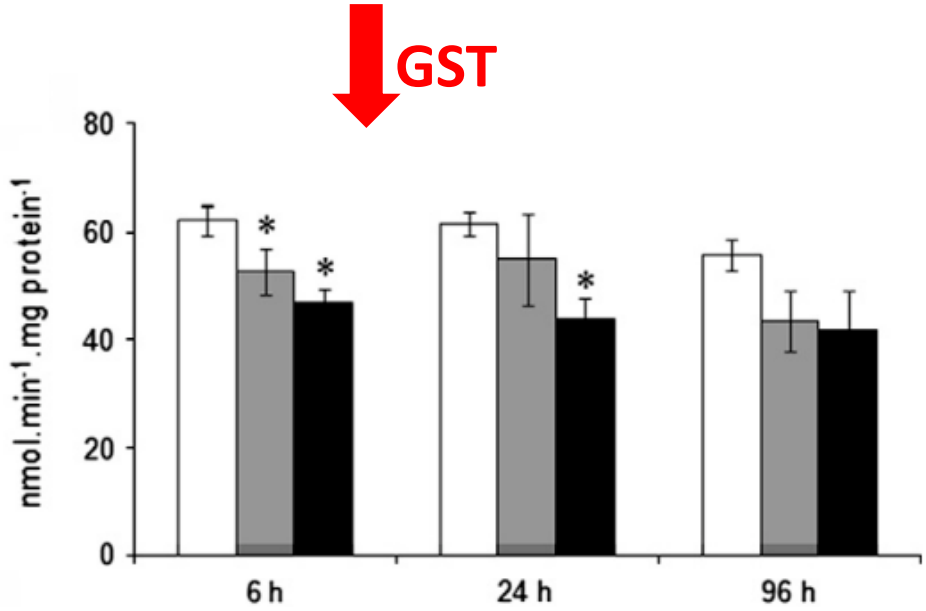
Effects of Roundup Transorb on fish: Hematology, antioxidant defenses and acetylcholinesterase activity

Kathya A. Modesto, Cláudia B.R. Martinez *

Juvenis de *Prochilodus lineatus* foram expostos ao Roundup Trasorb®

Roundup Transorb: 1 e 5 mg L^{-1}

∆t: 6, 24 e 96 h

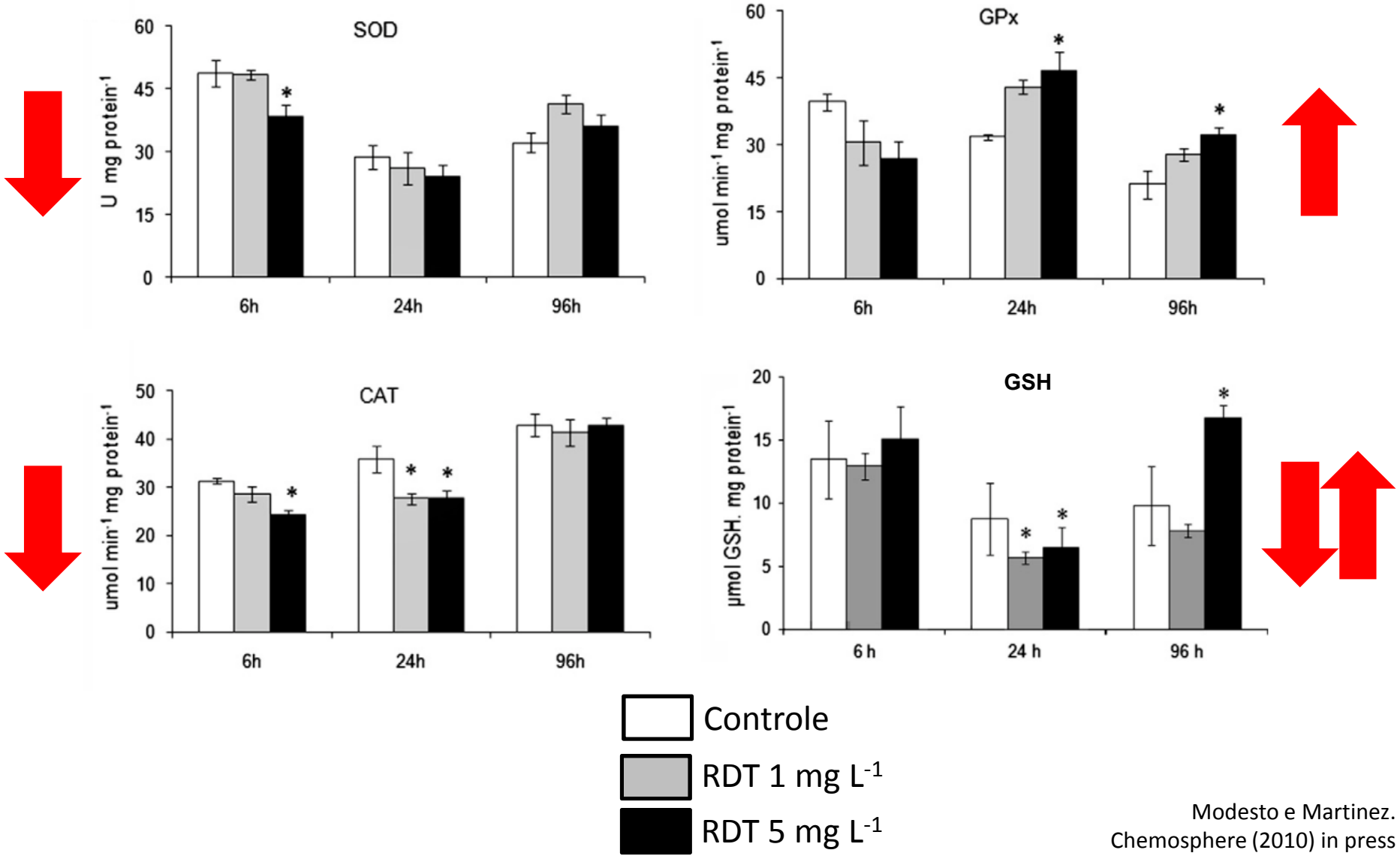


Controle

RDT 1 mg L^{-1}

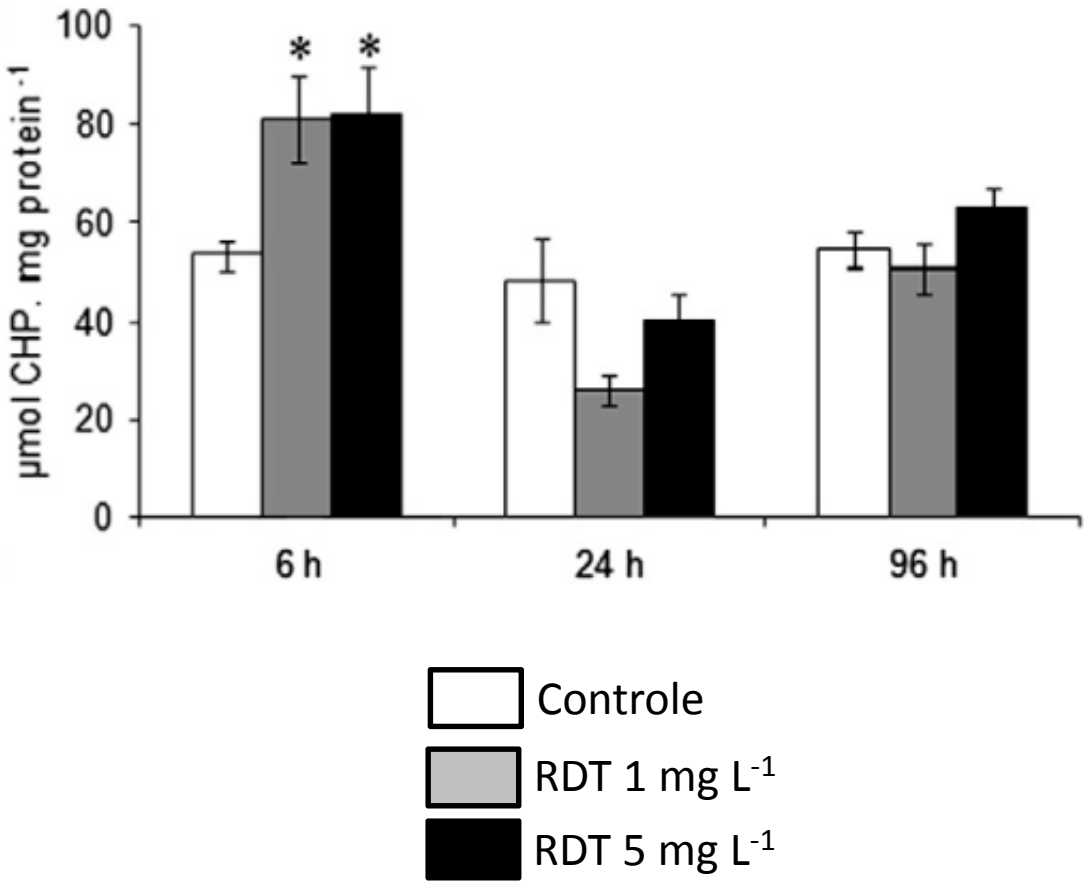
RDT 5 mg L^{-1}

DEFESAS ANTIOXIDANTES

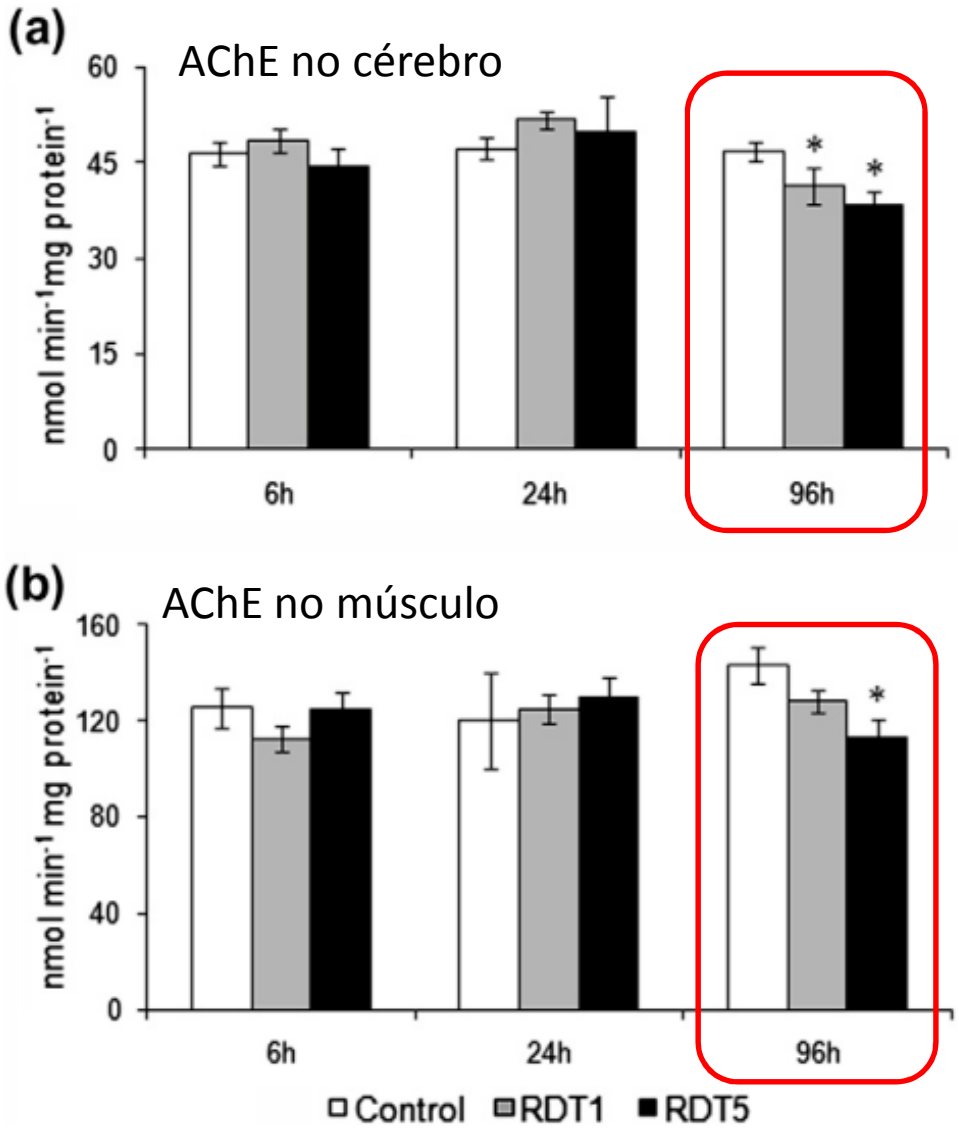


Modesto e Martinez.
Chemosphere (2010) in press

Peroxidação lipídica apenas no menor tempo de exposição (6 h)



Modesto e Martinez.
Chemosphere (2010) in press



Controle

RDT 1 mg L^{-1}

RDT 5 mg L^{-1}

Roundup Transorb: também promoveu inibição da acetilcolinesterase no cérebro e músculo de *Prochilodus lineatus*.

Modesto e Martinez.
Chemosphere (2010) in press

Roundup Transorb

Avaliação de danos no DNA

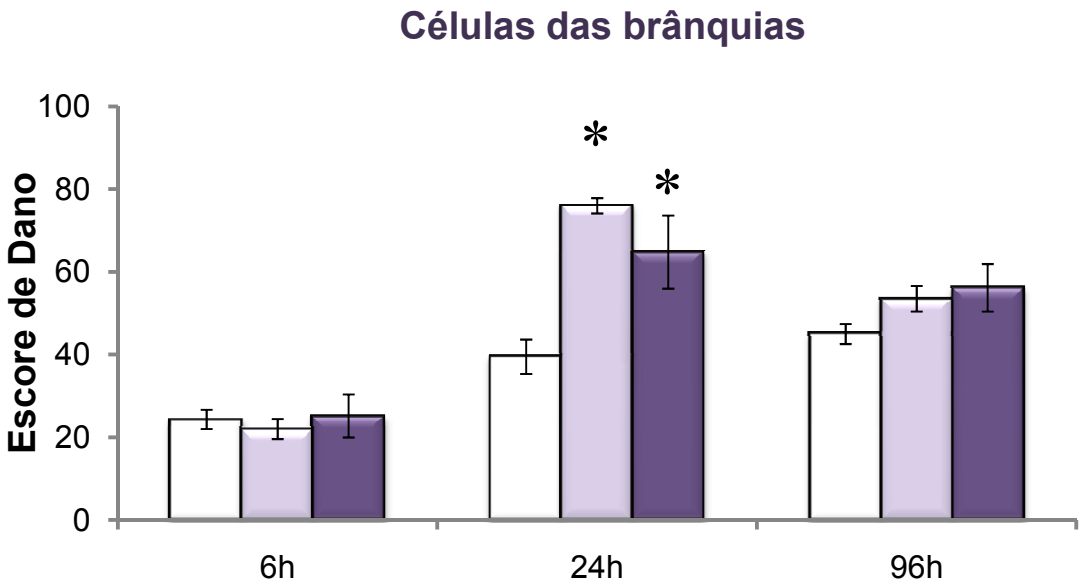
Ensaio do Cometa

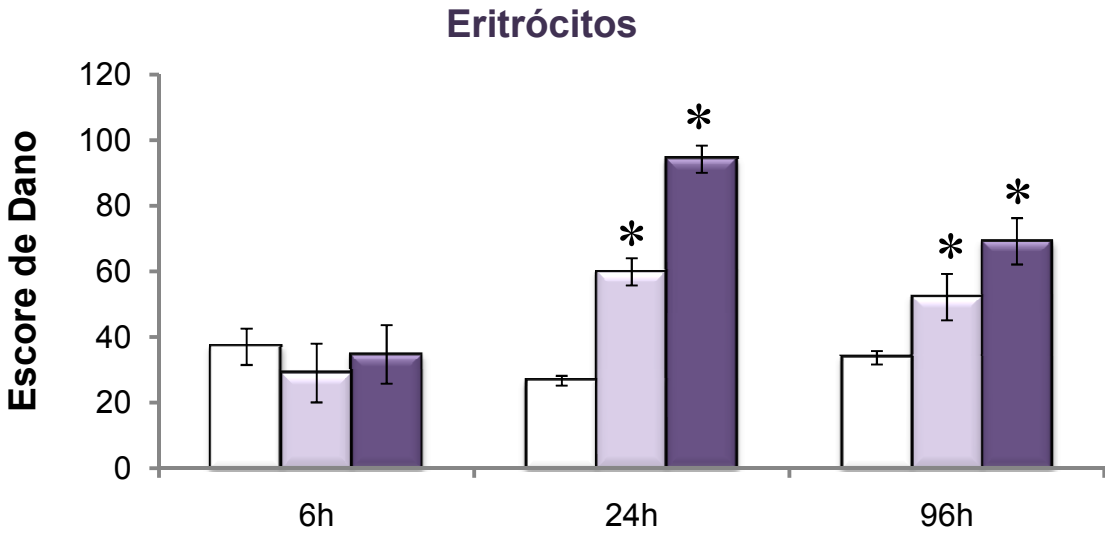
Exposição *in vivo*

Controle

RDT 1 mg L^{-1}

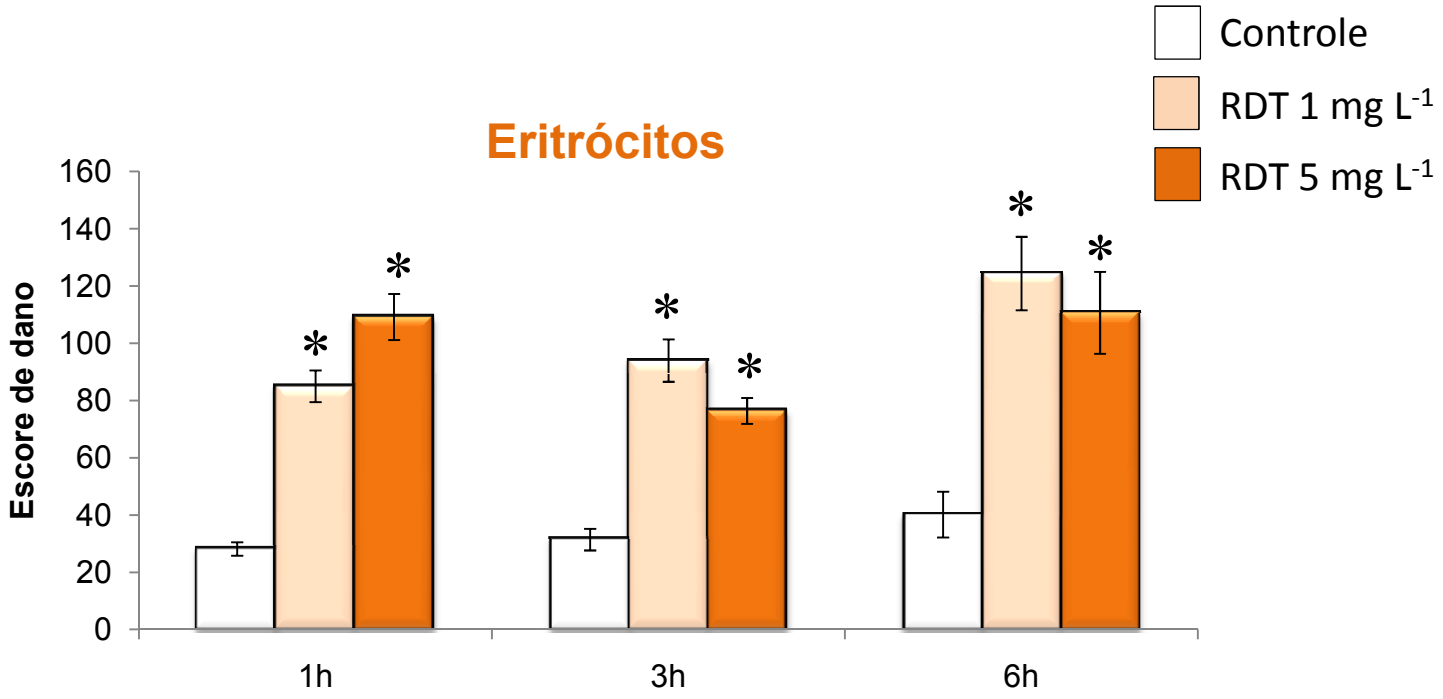
RDT 5 mg L^{-1}





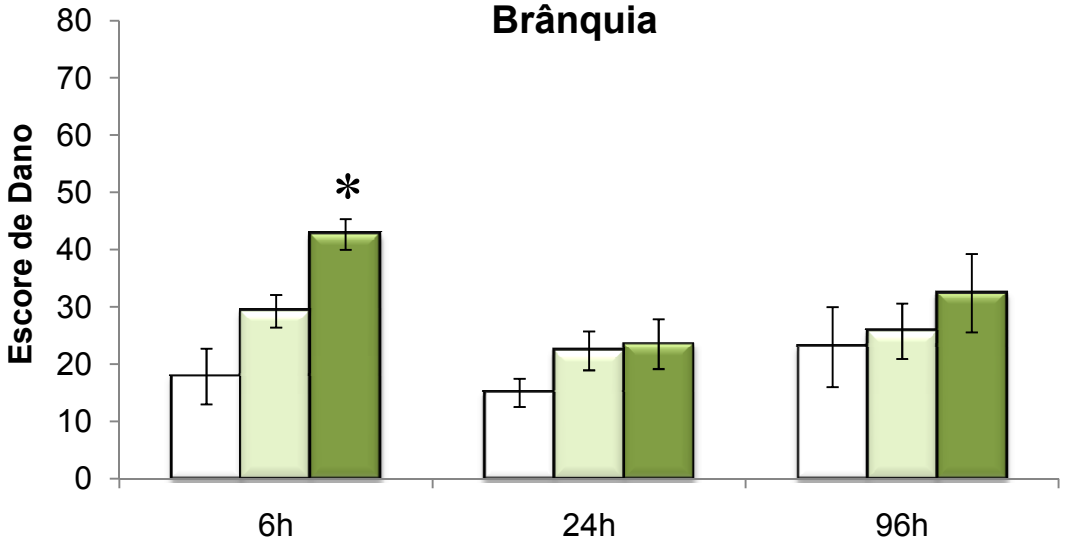
Roundup Transorb

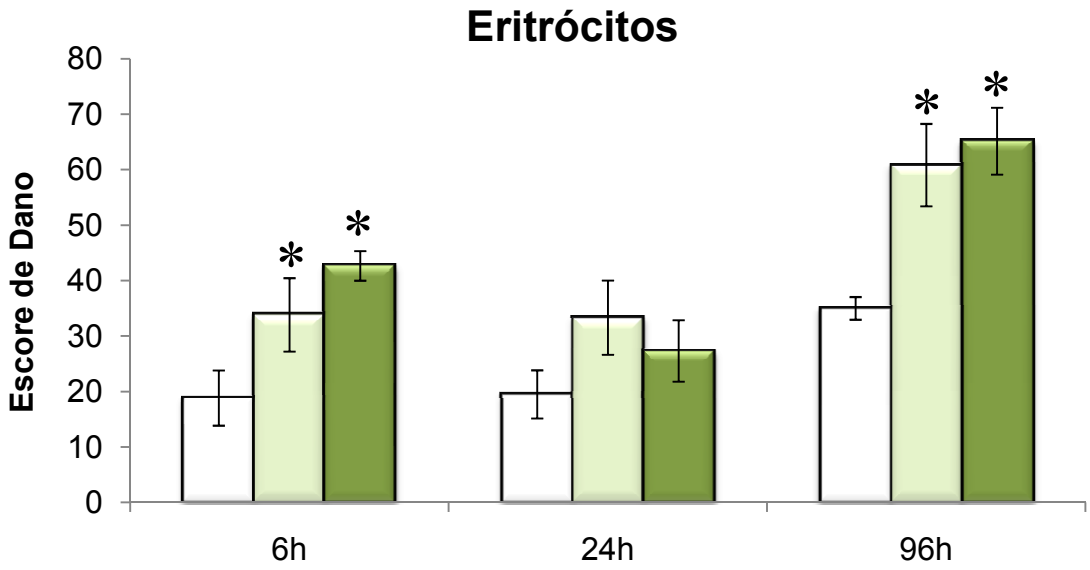
Avaliação de danos no DNA → **Ensaio do Cometa Exposição *in vitro***



Roundup Transorb promoveu danos no DNA de eritrócitos expostos diretamente ao herbicida, *in vitro*, em todos os tempos de exposição testados (1, 3 e 6h)

GLIFOSATO





Exposições *in vivo*

Juvenis de *Prochilodus lineatus* foram expostos apenas ao glifosato

- Controle
- Glifosato 1 mg L^{-1}
- Glifosato 5 mg L^{-1}

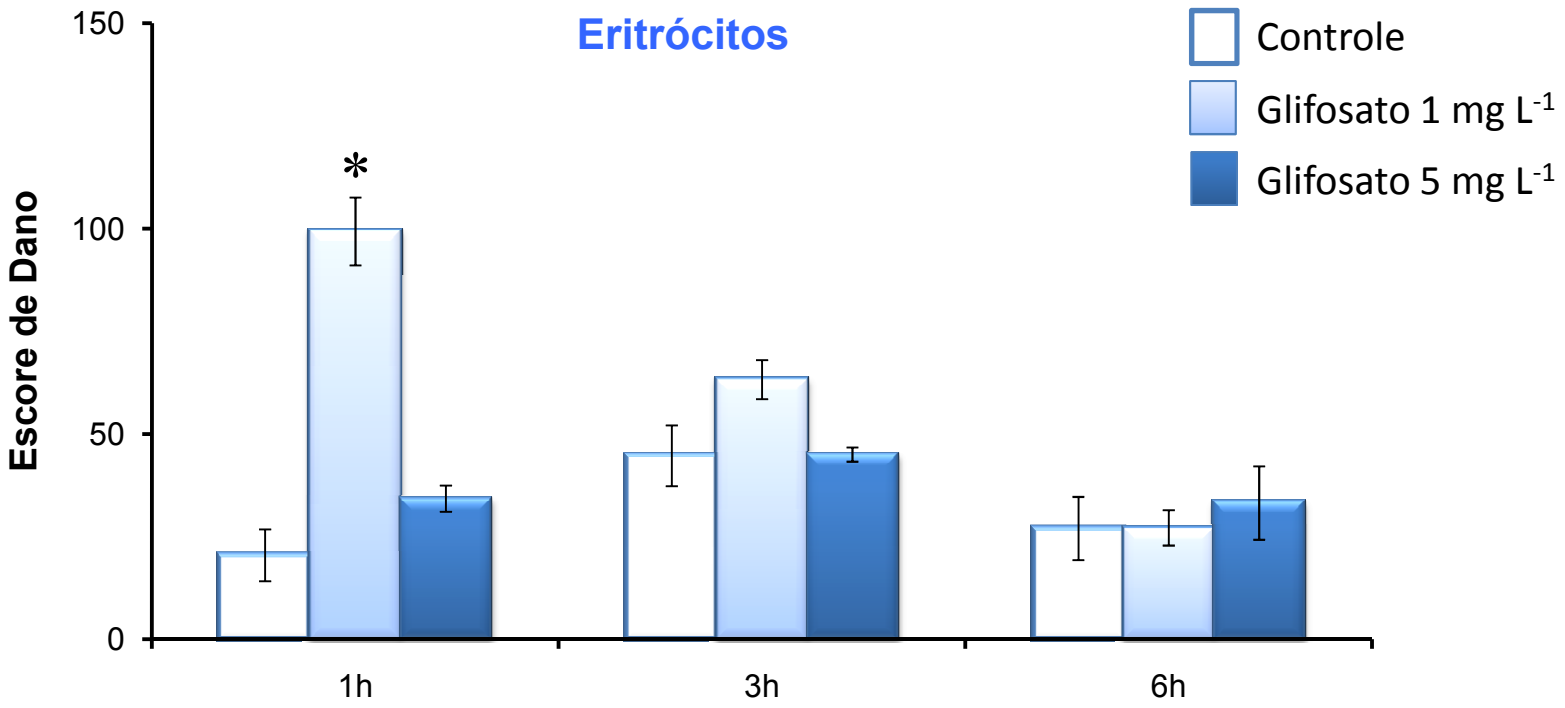
Glifosato

↓

Promove danos no DNA de células branquiais e de eritrócitos

Glifosato

Avaliação de danos no DNA → **Ensaio do Cometa**
Exposição *in vitro*

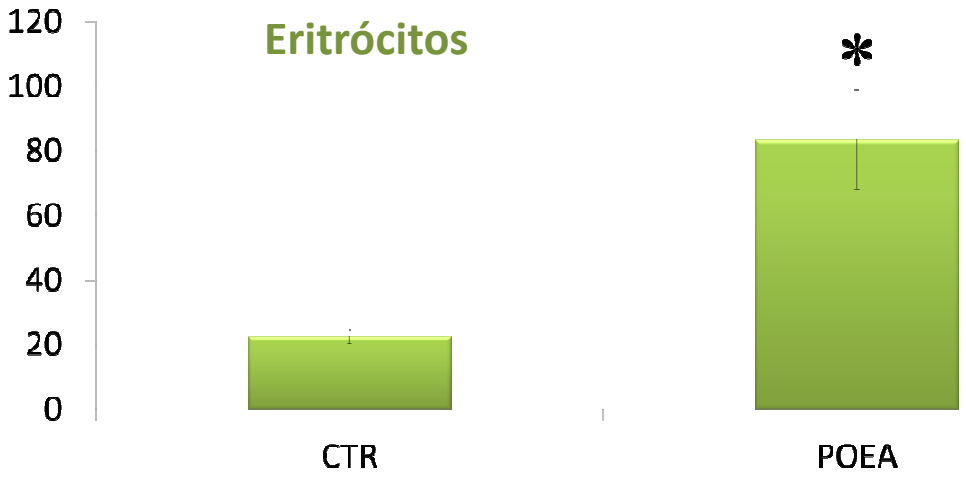


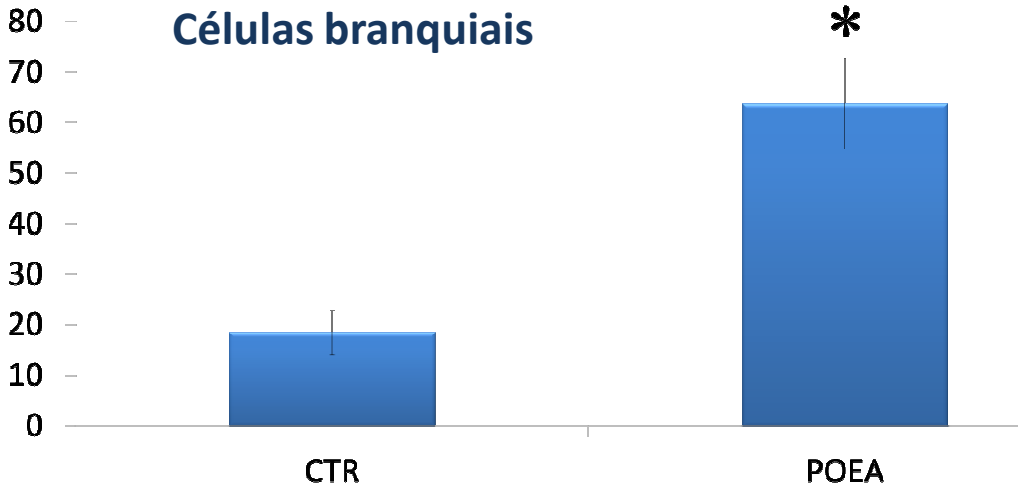
Glifosato promoveu danos no DNA de eritrócitos expostos diretamente ao herbicida, *in vitro*, apenas após 1 h de exposição a menor concentração testada

Roundup

Glifosato + Surfactante

O polioxietilenoamino (POEA) é o surfactante usado na formulação de herbicidas a base de glifosato, como o Roundup Original.





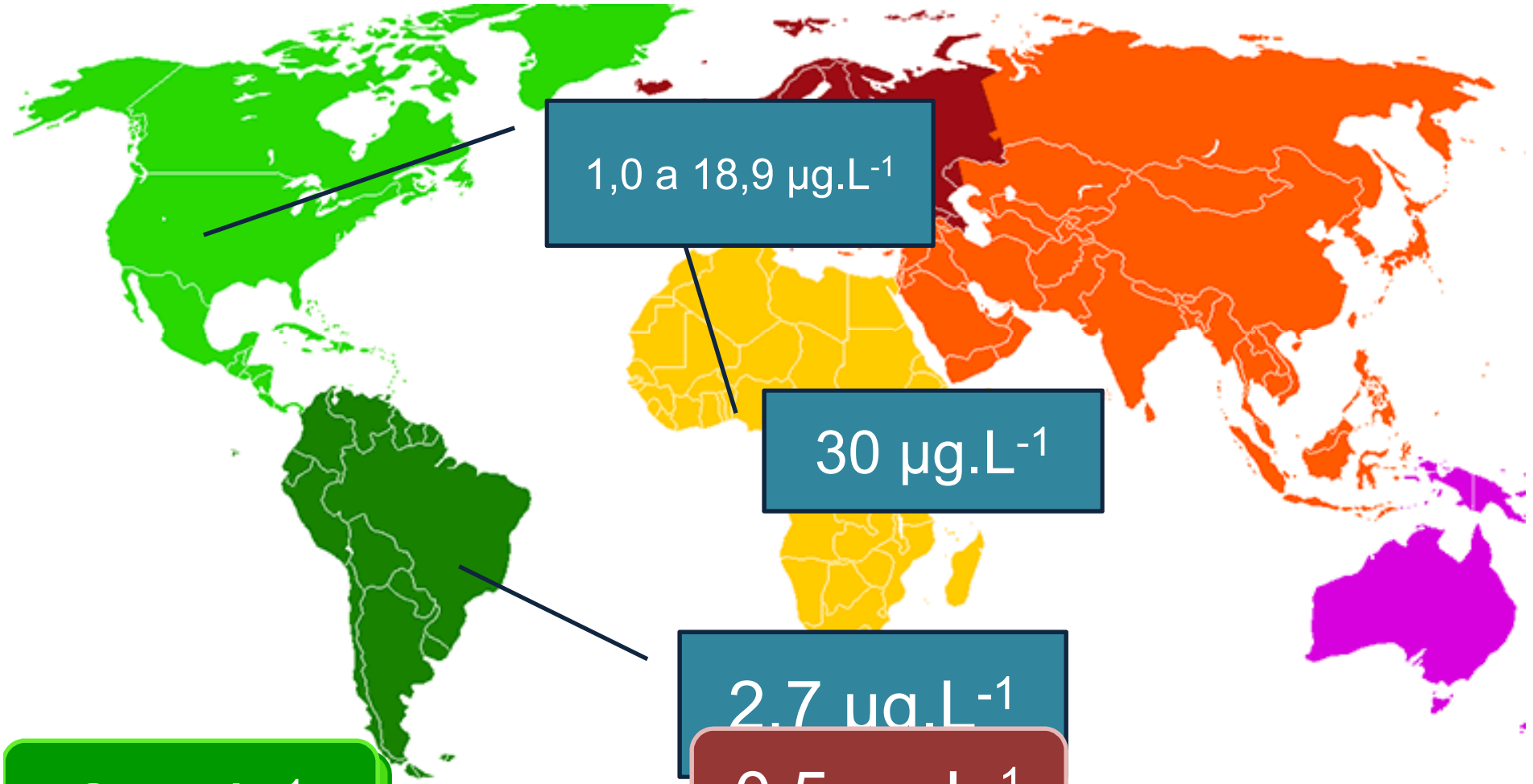
Escores danos no DNA em eritrócitos e células branquiais de *P. lineatus* expostos apenas à água (CTR) ou a 4 mg.L^{-1} de POEA, **durante 24 h**. As barras representam a média e a linha vertical o erro padrão. * diferente do CTR ($p < 0,01$).

ATRAZINA

- cana-de-açúcar, milho, soja, entre outras

- introduzida na agricultura na década de 50
- consumo mundial → 70.000 a 90.000 t/ano
- consumo no Brasil: segundo herbicida mais consumido

Contaminação Aquática por Atrazina



(CEREJEIRA et al., 2003; KONSTANTINOU et al., 2006; (ARMAS et al., 2007) (DAVI et al., 2007)

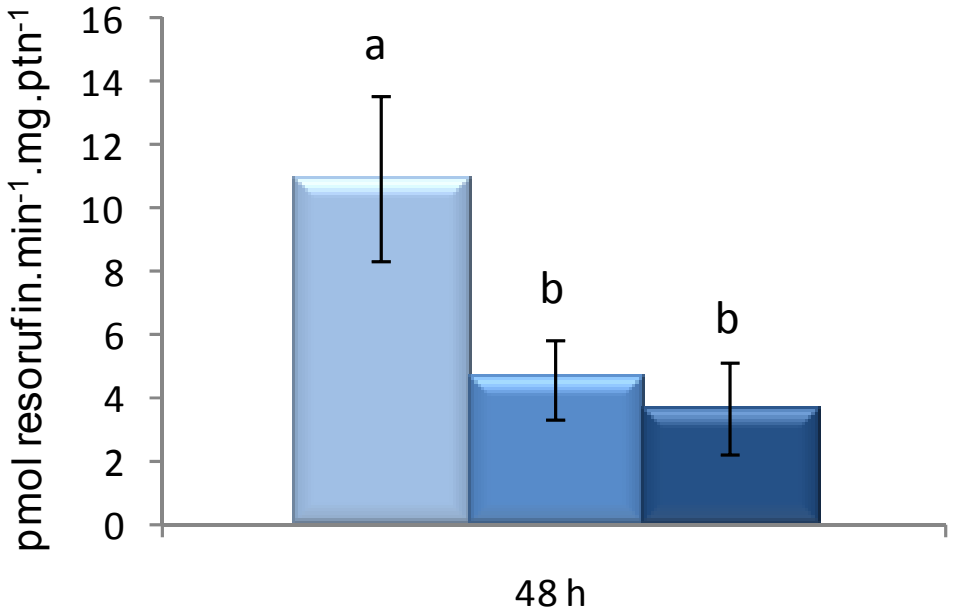
Efeitos da atrazina para *Prochilodus lineatus:* genotoxicidade e defesas antioxidantes

Peixes jovens expostos ao herbicida por 48 e 96 h

Duas [ATRAZINA]

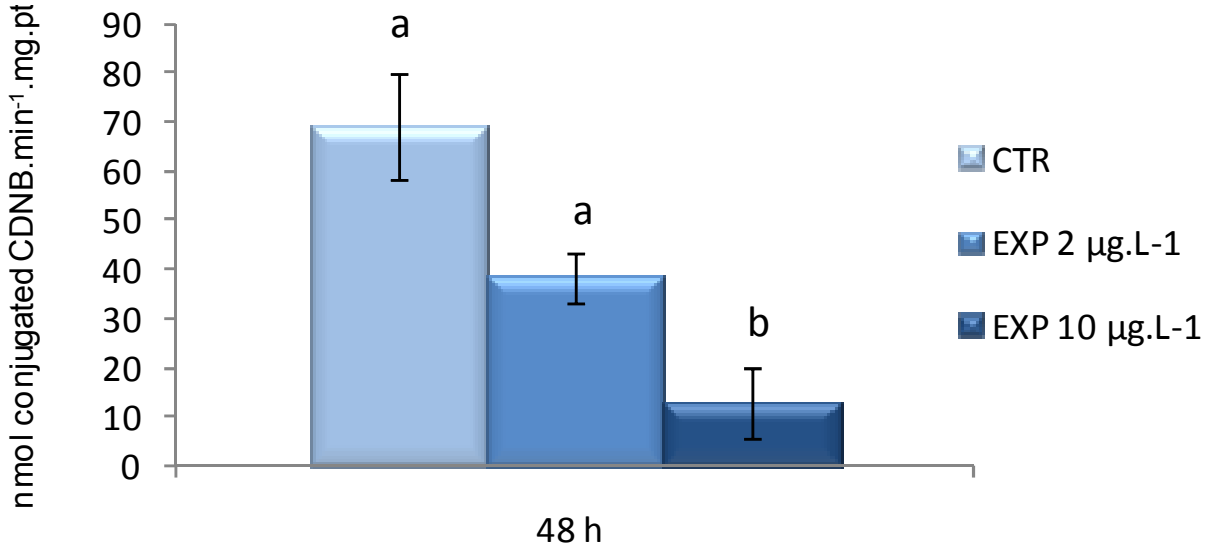
- **2 µg.L^{-1} -** Concentração limite definida pelo CONAMA (2005)
- **10 µg.L^{-1} –** corresponde à metade da concentração indicada para uso na agricultura

EROD

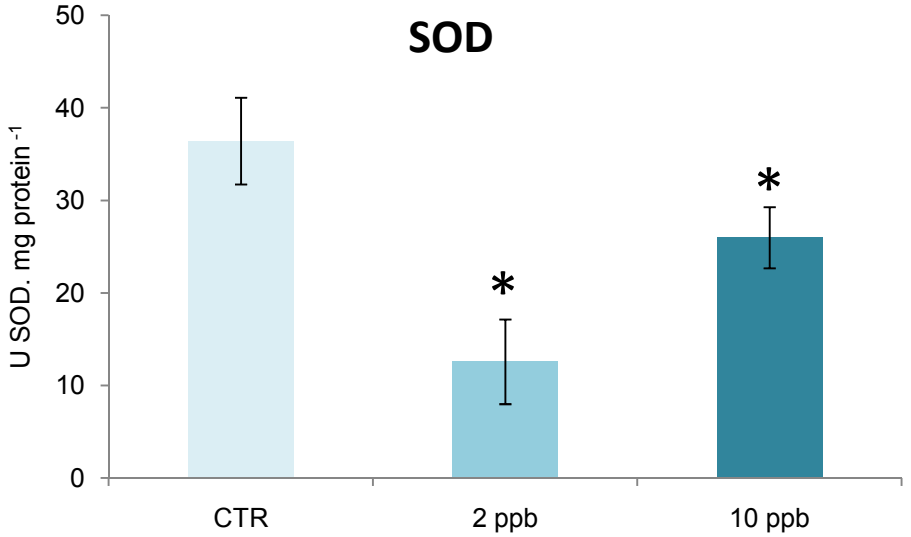


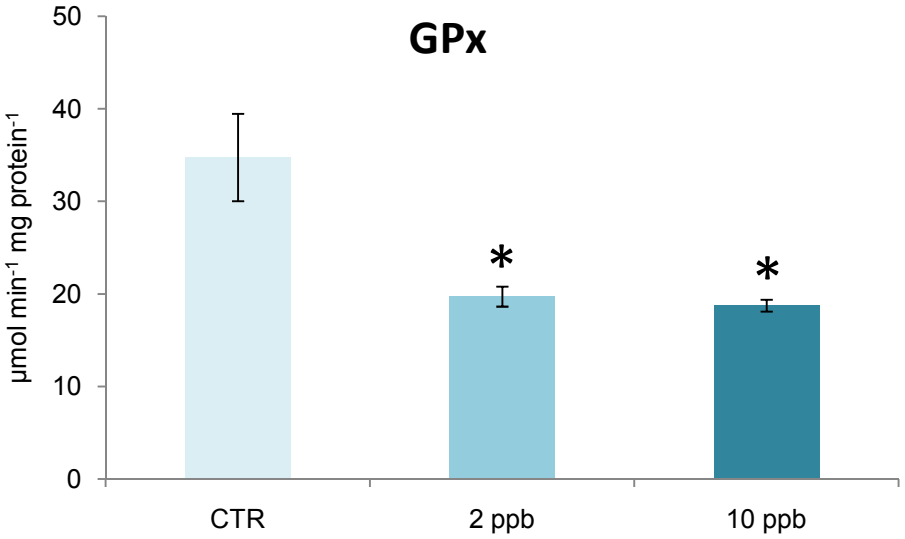
Atrazina inibiu as vias de biotransformação

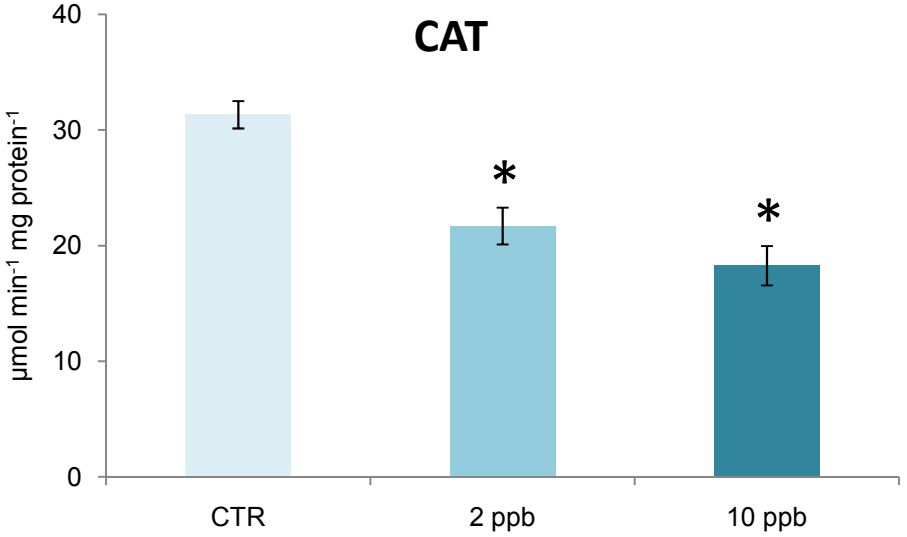
GST



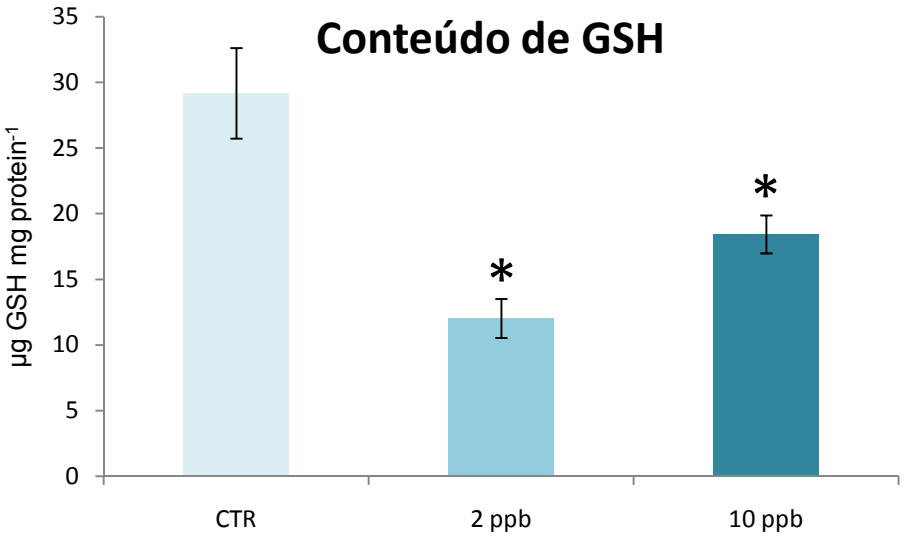
Enzimas antioxidantes primárias - FÍGADO





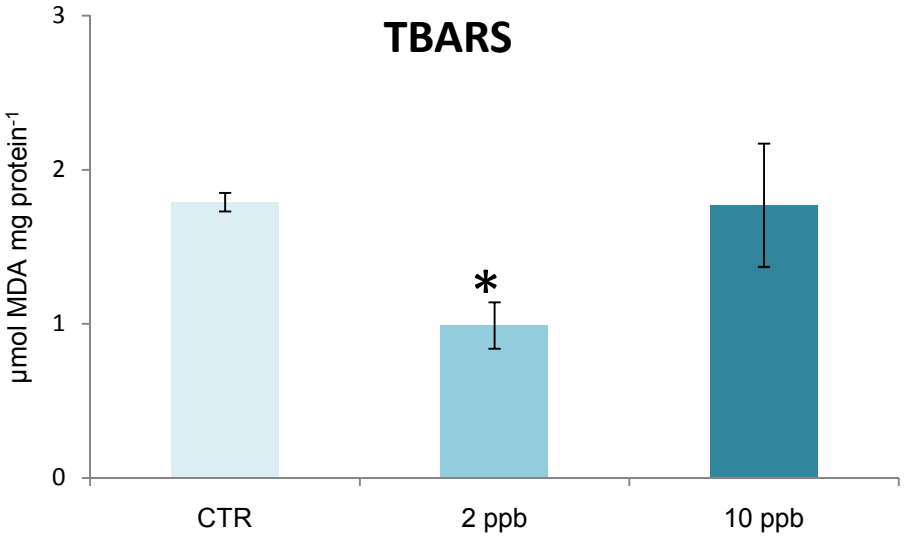


Atrazina inibiu SOD, CAT e GPx



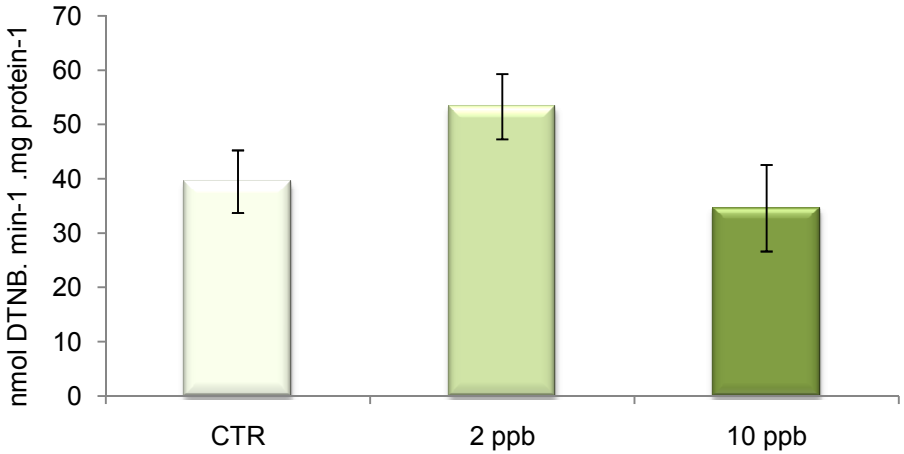
Atrazina promoveu redução significativa do conteúdo de GSH no fígado

Não houve indicação de peroxidação lipídica pelo ensaio TBARS



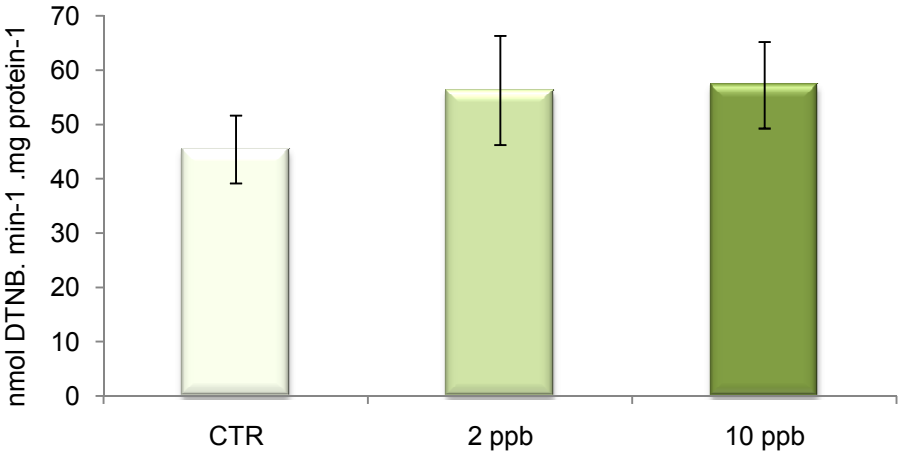
Peixes jovens (13 g) expostos a atrazina por 48 h

AChE cérebro

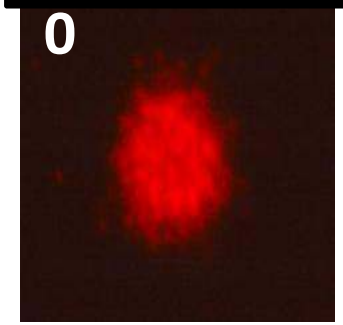


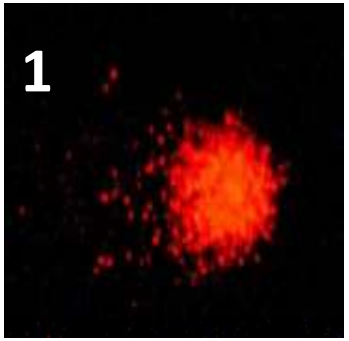
Atrazina não interferiu na atividade da acetilcolinesterase

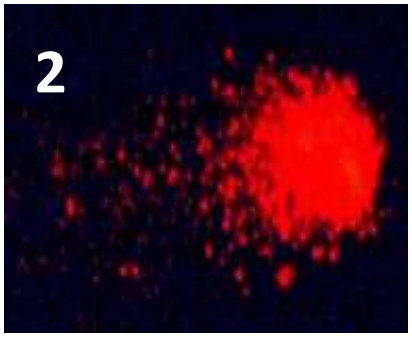
AChE músculo

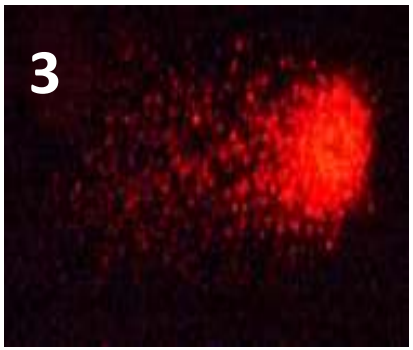


Escore de dano

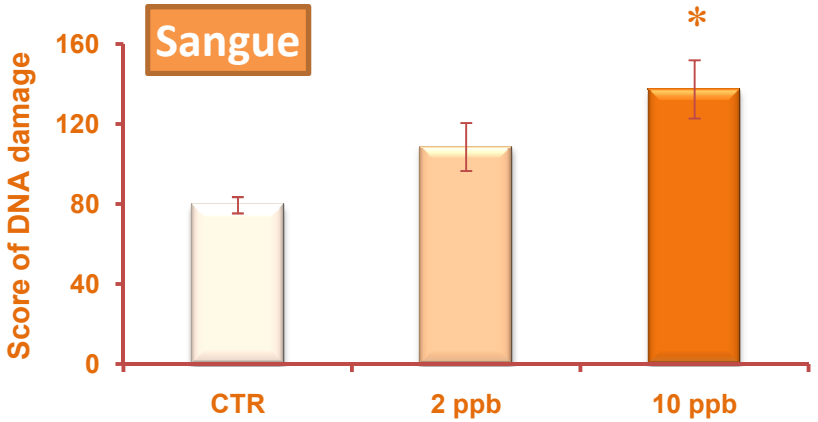


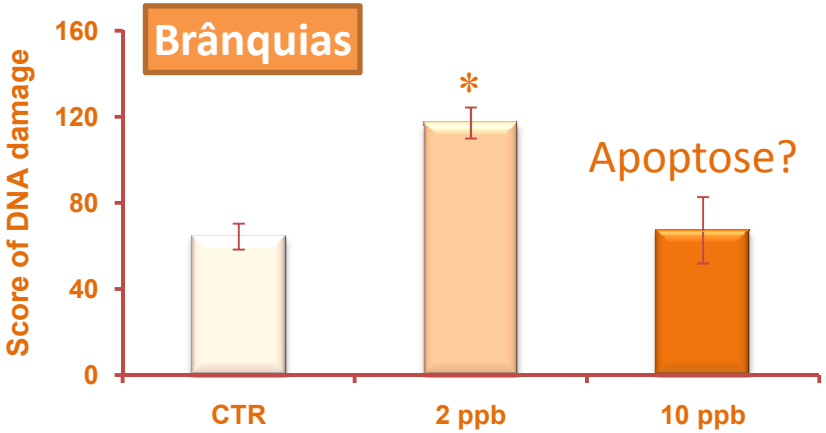




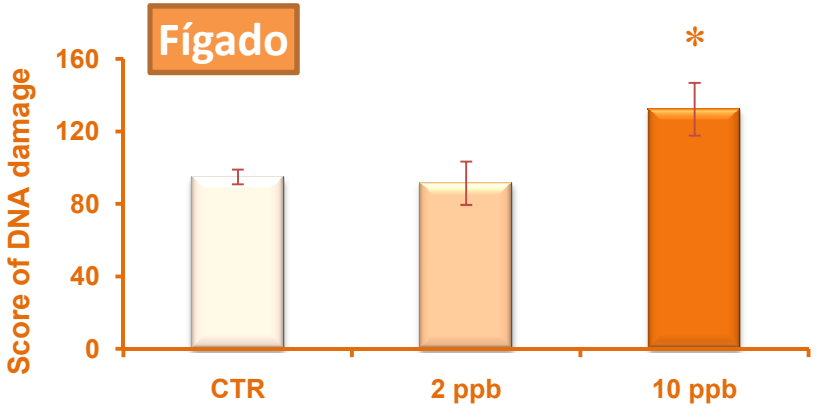


Danos no DNA em peixes expostos a atrazina por 48 h





Apoptose?



HERBICIDA	BIOMARCADORES				
	BIOQUÍMICOS			GENOTÓXICOS Danos DNA – Teste do Cometa	
	Biotrans	**Estresse Oxidativo**	**AChE**	***In vivo***	***In vitro***
GLIFOSATO	-	-	-	↑	↑
ROUNDUP	↑ GST	↓SOD, ↓CAT, = GPx, ↑ GSH ↑LPO	↓	↑	↑
ROUNDUP TRANSORB	↓ GST	↓SOD, ↓CAT, ↑GPx, ↓↑ GSH ↑LPO	↓	↑	↑
POEA	-	-	-	↑	-
ATRAZINA	↓ EROD; ↓ GST	↓SOD, ↓CAT, ↓GPx, ↓ GSH ↓LPO	=	↑	-

OBRIGADA!