Dipartimento Scienze della Vita – Seconda Università di Napoli – Caserta - ITALY

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Aspetti ecotossicologici e valutazione del rischio da esposizione a farmaci e loro derivati biotici e/o abiotici

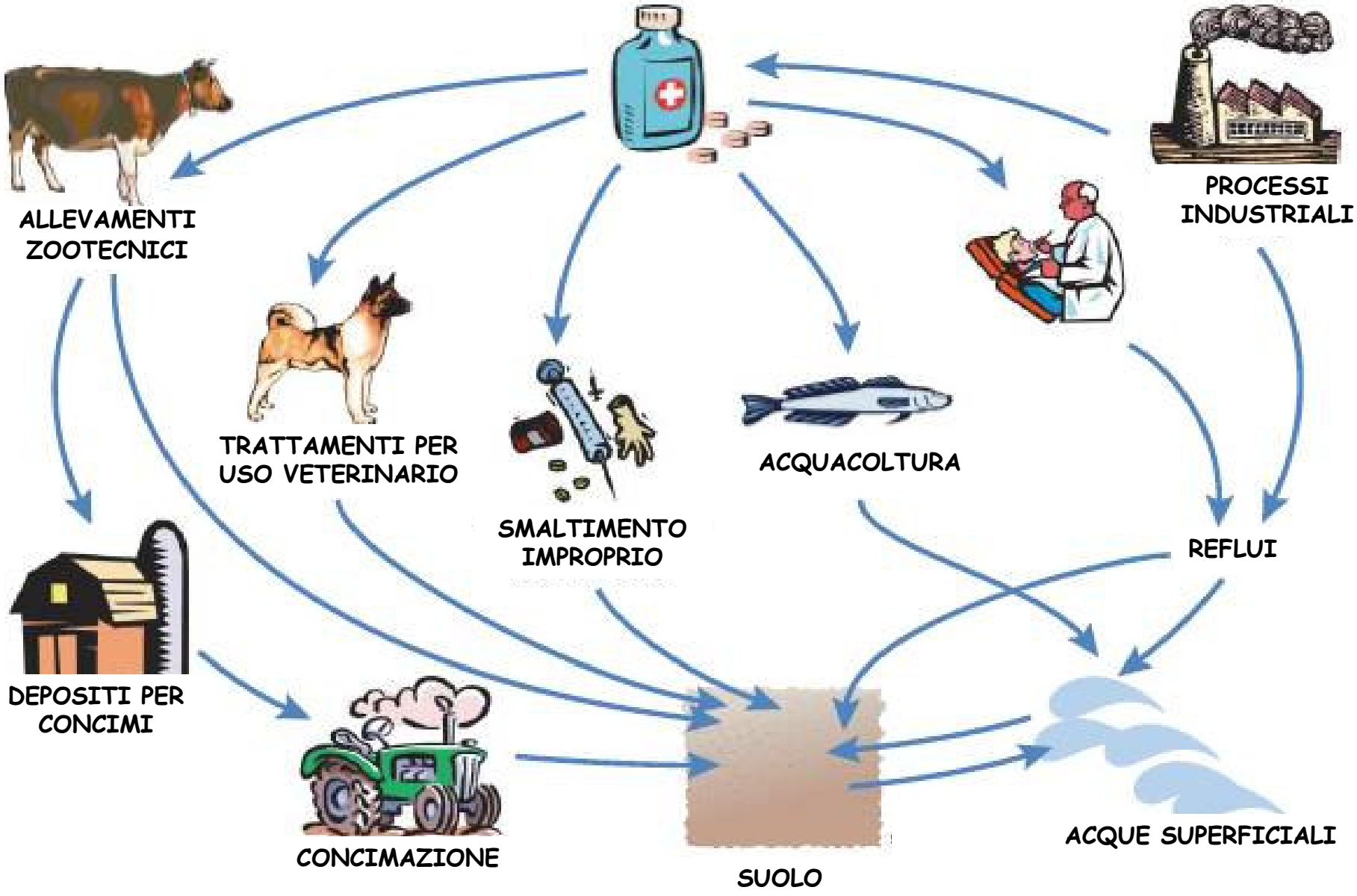
ISPRA
Istituto Superiore per la Protezione e la Ricerca Ambientale

Giornate di studio
20-22 ottobre 2010

CENTRO INTERUNIVERSITARIO BIOLOGIA MARINA ED ECOLOGIA APPLICATA

I FARMACI:

nuova classe di inquinanti ambientali



Stima del rischio

$$R = \frac{PEC}{PNEC}$$

PEC : massime concentrazioni ambientali negli effluenti (PECeffl) e acque di superficie (PECsup) ottenute dalla letteratura

PNEC : concentrazione che non dà effetto tossico

$R \geq 1$ ⇒ rischio ambientale

European Medicines Agency

European Medicines Agency

$$PEC_w = \frac{A \times (100 - R)}{365 \times P \times V \times D \times 100}$$

A = consumo annuo Kg/a

R= rimozione in %

P = numero abitanti

V = volume acque reflue pro capite (0.2 m^3)

D = fattore di diluizione in ambiente (default parei a 10)

The PNEC data in the fass.se system

Data should reflect tests performed on 3 trophic levels; algae, crustaceans and fish.

In order to ensure acceptable safety margin when going from lab tests on *x* individuals to an entire population in the environment; a safety factor (AF) is applied. Thus;

$$PNEC = \frac{EC_{50} / LC_{50}}{AF}$$

If only short term acute data available then **AF = 1000**

If chronic long-term data are available from all 3 trophic levels, then **AF = 10**

European Medicines Agency

Conclusions under the current Directive are:

1. **PEC / PNEC < 1 = No immediate concern**
2. **PEC / PNEC 1 - 10 = Of concern if supply volumes increase**
3. **PEC / PNEC 10 - 100 = Further data required**
4. **PEC / PNEC > 100 = Reduce risk immediately**

Istituto Mario Negri: da Ettore Zuccato, 2003

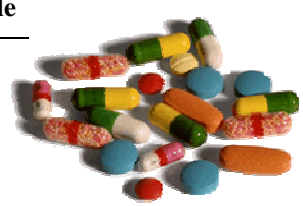
Impianti acque	ng/l								
Farmaci	Cagliari	Cosenza	Palermo	Roma	Napoli pre	Napoli post	Torino	Varese lago	Varese olona
Penicilline									
Amoxicillina	7,40	nd	120,35	15,20	nd	nd	4,74	4,68	25,26
Chinoloni									
Ciprofloxacina	146,30	26,60	178,80	91,00	1121,00	251,00	514,53	378,30	322,00
Ofloxacina	600,00	150,00	474,40	346,88	2842,50	1081,00	864,50	964,00	738,00
Macrolidi-lincosamidi									
Claritromicina	59,40	7,82	18,06	12,60	95,47	72,80	15,20	12,00	52,10
Spiramicina	75,00	1,41	129,40	59,20	22,00	161,00	11,32	18,60	91,30
Lincomicina	28,00	43,05	639,00	25,24	303,40	846,00	30,50	11,00	21,60
Eritromicina	161,00	27,40	63,66	19,84	nd	353,44	27,20	47,44	90,00
Sulfamidici									
Sulfamethoxazole	96,60	46,20	127,17	110,21	356,10	317,00	230,00	212,30	253,15
Diuretici									
Furosemide	585,00	25,60	560,00	289,50	39,80	601,60	2101,64	1177,82	649,80
Idroclorotiazide	430,60	60,27	654,00	261,20	267,60	255,65	1253,27	986,23	877,00
Cardiovascolari									
Atenololo	254,43	27,30	260,00	69,64	619,30	955,14	1168,00	554,30	466,00
Bezafibrato	9,18	0,33	7,55	15,35	100,00	116,70	54,80	55,05	87,00
Vari									
Ranitidina	263,00	36,24	260,00	76,80	870,00	610,00	516,88	335,50	288,20
Omeprazolo	nd	nd	nd	nd	nd	nd	nd	nd	nd
Salbutamolo	10,45	1,06	9,30	6,46	14,24	18,48	11,27	5,70	5,64
Diazepam	nd	nd	nd	nd	nd	nd	nd	nd	nd
Enalapril	nd	nd	nd	nd	nd	nd	nd	nd	nd
Metotressato	nd	nd	nd	12,62	nd	nd	nd	nd	nd
Ciclofosfamide	2,06	nd	0,51	0,50	nd	nd	9,00	0,74	0,56
Acido clofibrico	nd	0,33	0,66	2,05	117,22	82,21	1,70	0,45	5,14
Demetildiazepam	4,72	1,02	3,80	3,56	90,00	61,80	nd	22,54	25,20
Oleandomicina	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ossitetraciclina	nd	nd	nd	nd	nd	nd	nd	nd	nd
Tilmicosina	nd	nd	nd	nd	nd	nd	nd	nd	nd
Tilosina	nd	0,04	0,08	nd	0,54	nd	0,50	0,93	nd

Istituto Mario Negri: da Ettore Zuccato, 2003

Impianti acque					
Farmaci	Mediane ng/L	Medie ng/L	Po Mediane	Po Max	Lambro
Penicilline					
Amoxicillina	4,74	19,74	0	0	0
Chinoloni					
Ciprofloxacina	251,00	336,61	0	26,2	14,4
Ofloxacina	738,00	895,70			
Macrolidi-lincosamidi					
Claritromicina	18,06	38,38	1,6	20,3	8,3
Spiramicina	59,20	63,25	9,8	43,8	74,2
Lincomicina	30,50	216,42	32,6	248,9	24,4
Eritromicina	47,44	87,78	3,2	15,9	4,5
Sulfamidici					
Sulfamethoxazole	212,30	194,30			
Diuretici					
Furosemide	585,00	670,08	3,5	67,2	254,7
Idroclorotiazide	430,60	560,65	4,6	24,4	255,8
Cardiovascolari					
Atenololo	466,00	486,01	17,2	41,7	241
Bezafibrato	54,80	49,55	1,9	2,7	57,2
Vari					
Ranitidina	288,20	361,85	1,3	4	38,5
Omeprazolo	0,00	0,00	0	0	0
Salbutamolo	9,30	9,18	1,1	1,7	2,5
Diazepam	0,00	0,00	0	0	0
Enalapril	0,00	0,00	0,05	0,12	0,5
Metotressato	0,00	1,40	0	0	0
Ciclofosfamide	0,51	1,49	0	0	0

Alcuni farmaci ritrovati nell'ambiente e loro biodegradabilità

Farmaci	Uso terapeutico	Concentrazioni nell'ambiente	Localizzazione	Bibliografia	Biodegradabilità
Aspirina	Analgesico	~1µg/l <50-1510	Acque di scarico Effluenti provenienti da vasche di sedimentazione	Richardson and Bowron (1985) Stumpf (1996)	Facilmente biodegradabile
Clofibrati	Ipolipemico	~40ng/l <0.5-1750ng/l <0.5-220ng/l <5-180ng/l <50-1560	Acqua di fiume Acqua di fiume (Berlino) Acqua di fiume (Europa) Acqua di fiume Effluenti provenienti da vasche di sedimentazione	Richardson and Bowron (1985) Heberer (1995)	Non degradabile
Eritromicina	Antibiotico	~1µg/l	Acqua di fiume	Watts et al. (1983)	Non degradabile
Estrogeni	Ormoni	0.2 to 0.5 nmol/l	Acqua di scarico, Tel Aviv, Israele	Shore et al. (1993)	Persistente
Estrogeni/estradiolo ed estrone	Ormoni	10µmol/giorno	Escreto giornalmente con le urine da donne incinte	Fostis (1987)	Persistente
Ibuprofen	Analgesico	Up to 12µg/l <5-41ng/l 17-139ng/l	Effluenti provenienti da vasche di sedimentazione Fiume Reno Altri fiumi	Stumpf et al. (1996)	Biodegradabile
Metotressato	Agente antineoplastico	~1µg/l <6.25ng/l <6.25ng/l	Acque di scarico Acqua di fiume Acqua potabile	Waggott (1981)	Persistente
Tetracicline	Antibiotici	~1µg/l	Acqua di fiume	Watts et al. (1983)	Non degradabile



Farmaci	Impianti di trattamento (MECeffl)	Acque di superficie (MECsup)	
AMLODIPINA	71 ng/L (Italia; Della Greca *et al.*, 2007)	-	Anti-ipertensivo
RANITIDINA	300-850 ng/L (Italia; Piredda e Carucci, 2005)	9,4 ng/L (Italia; Zuccato, 2000)	Protettore gastrico

Farmaci	Impianti di trattamento (MECeffl)	Acque di superficie (MECsup)
BEZAFIBRATO	0,91 µg/L (Italia; Andreozzi *et al.*, 2003)	57 ng/L (Italia, Lambro - Zuccato *et al.*, 2005) 0,002 µg/L (Italia, Po; Castiglioni *et al.*, 2004)
FENOFIBRATO	0,16 µg/L (Italia; Andreozzi *et al.*, 2003) 110 ng/L (Spagna; Hernando *et al.*, 2006)	
GEMFIBROZIL	4,76 µg/L (Italia; Andreozzi *et al.*, 2003) 250,09 ng/L (Italia; Zuccato *et al.*, 2006) 2376 ng/L (Spagna; Hernando *et al.*, 2006)	
SILDENAFIL CITRATO	5,25 ng/L (Italia; Zuccato *et al.*, 2006)	

Ipolipemizzanti

Ritrovamenti ambientali

TAMOXIFENE (antitumorale e antiestrogeno)

71 ng/L

(Thomas e Hilton, 2004)

20-40 ng/L

(Ashton *et al.*, 2004)

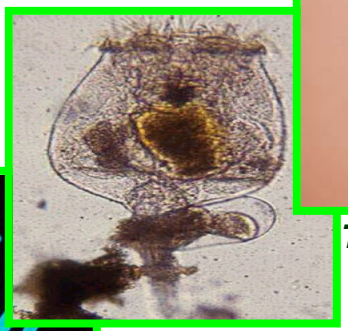
Ritrovamento in acque superficiali

Antibiotici

Eritromicina	Ossitetraciclina	Sulfametossazolo	Ofloxacina	Lincomicina	Claritromicina
0.02 µg/L (Germania, Hirsh et al.,1999)	**0.05 µg/L** (Germania,Hirsh et al.,1999)	**0.02 µg/L** (Germania,Hirsh et al.,1999)	**0.05 µg/L** (Germania Kummerer et al., 2000)	**0.08 µg/L** (Italia,Castiglioni et al., 2004)	**0.02 µg/L** (Germania, Hirsh et al.,1999)
0.016 µg/L (Italia,Calamari et al.,2003)	**0.019 µg/L** (Italia,Calamari et al.,2003)	-	-	-	**0.020 µg/L** (Italia,Calamari et al.,2003)

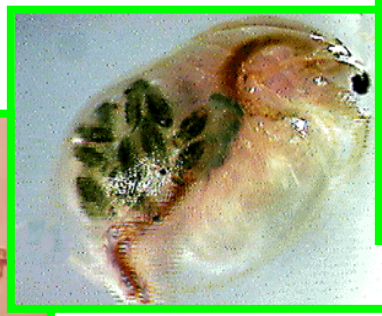
TOSSICITA'
ACUTA

Vibrio fischeri
ISO 11348-3

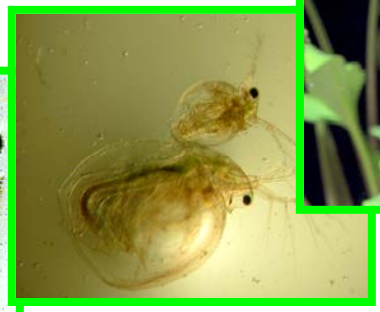


Brachionus calyciflorus
ASTM E1440-91

Thamnocephalus platyurus



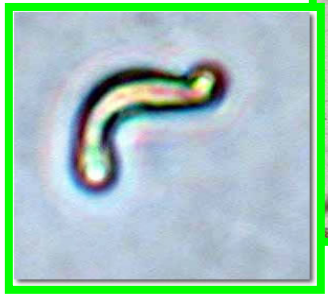
Daphnia magna
ISO 6341



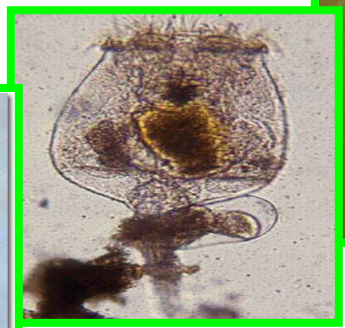
Ceriodaphnia dubia
ISO/CD 20665

Danio rerio
ISO/DIS 7346-1

TOSSICITA'
CRONICA



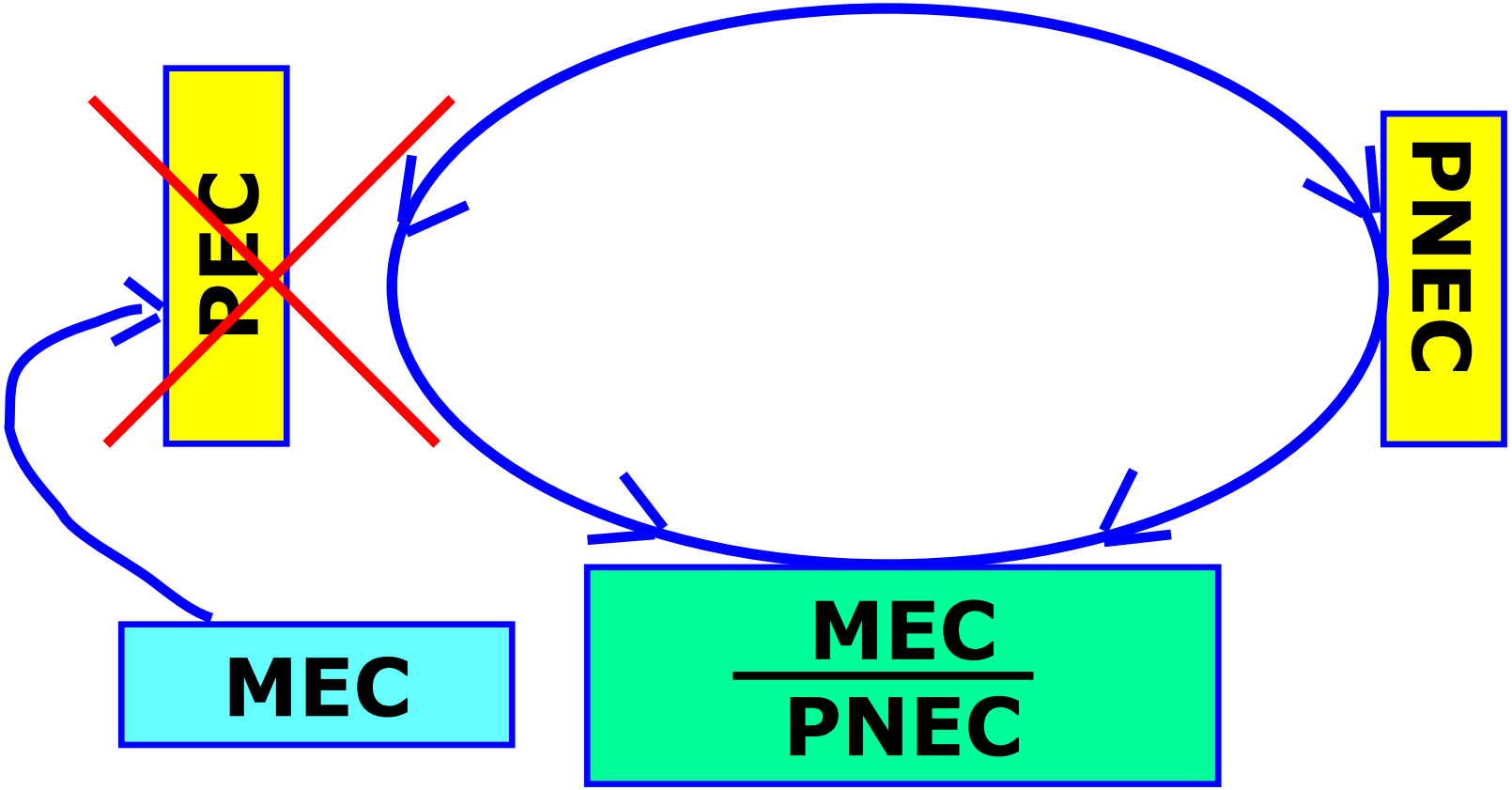
Pseudokirchneriella subcapitata
ISO 8692



Brachionus calyciflorus
ASTM E1440-91

Ceriodaphnia dubia
ISO/CD 20665

RISCHIO AMBIENTALE



Stima del rischio

$$R = \frac{MEC}{PNEC}$$

MEC : massime concentrazioni ambientali negli effluenti (MECeffl) e acque di superficie (MECsup) ottenute dalla letteratura

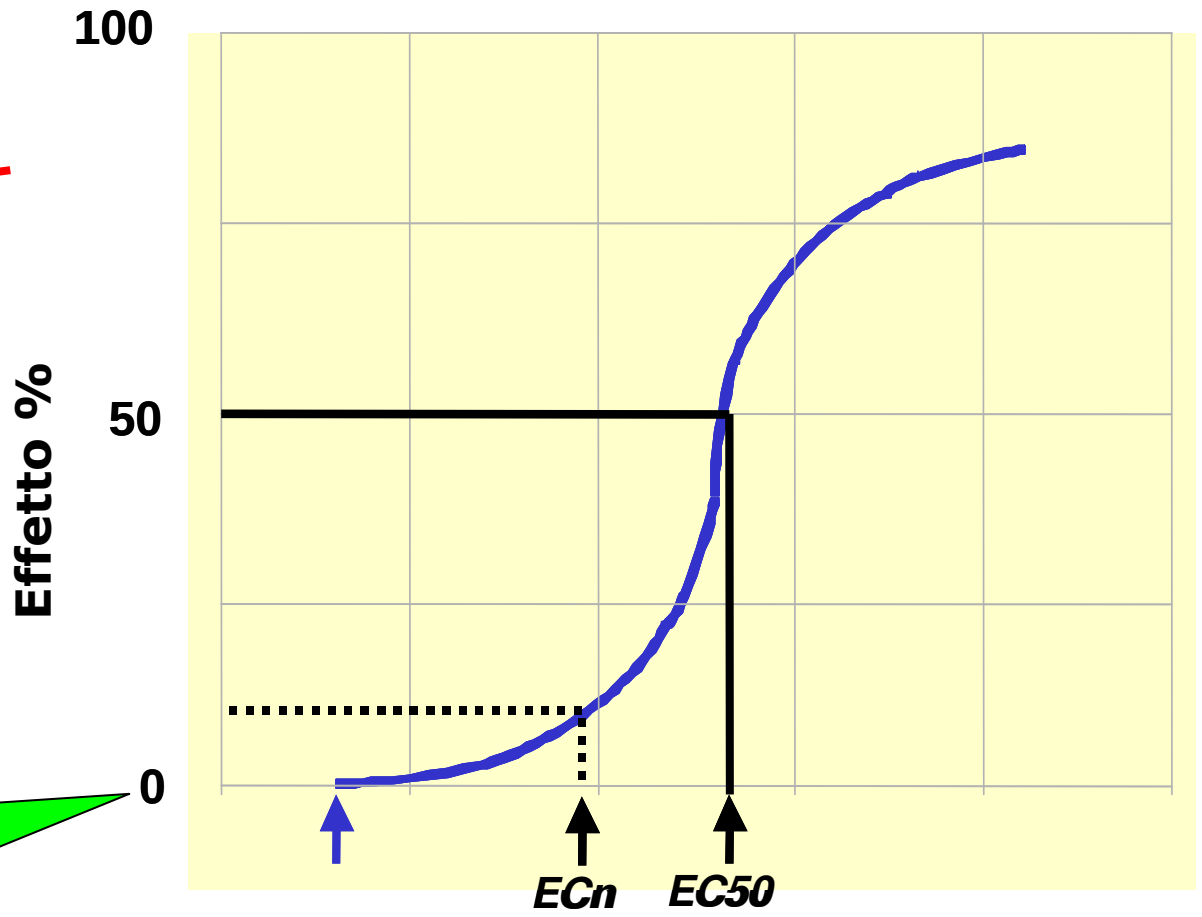
PNEC : concentrazione che non dà effetto tossico

$R \geq 1$ ⇒ rischio ambientale

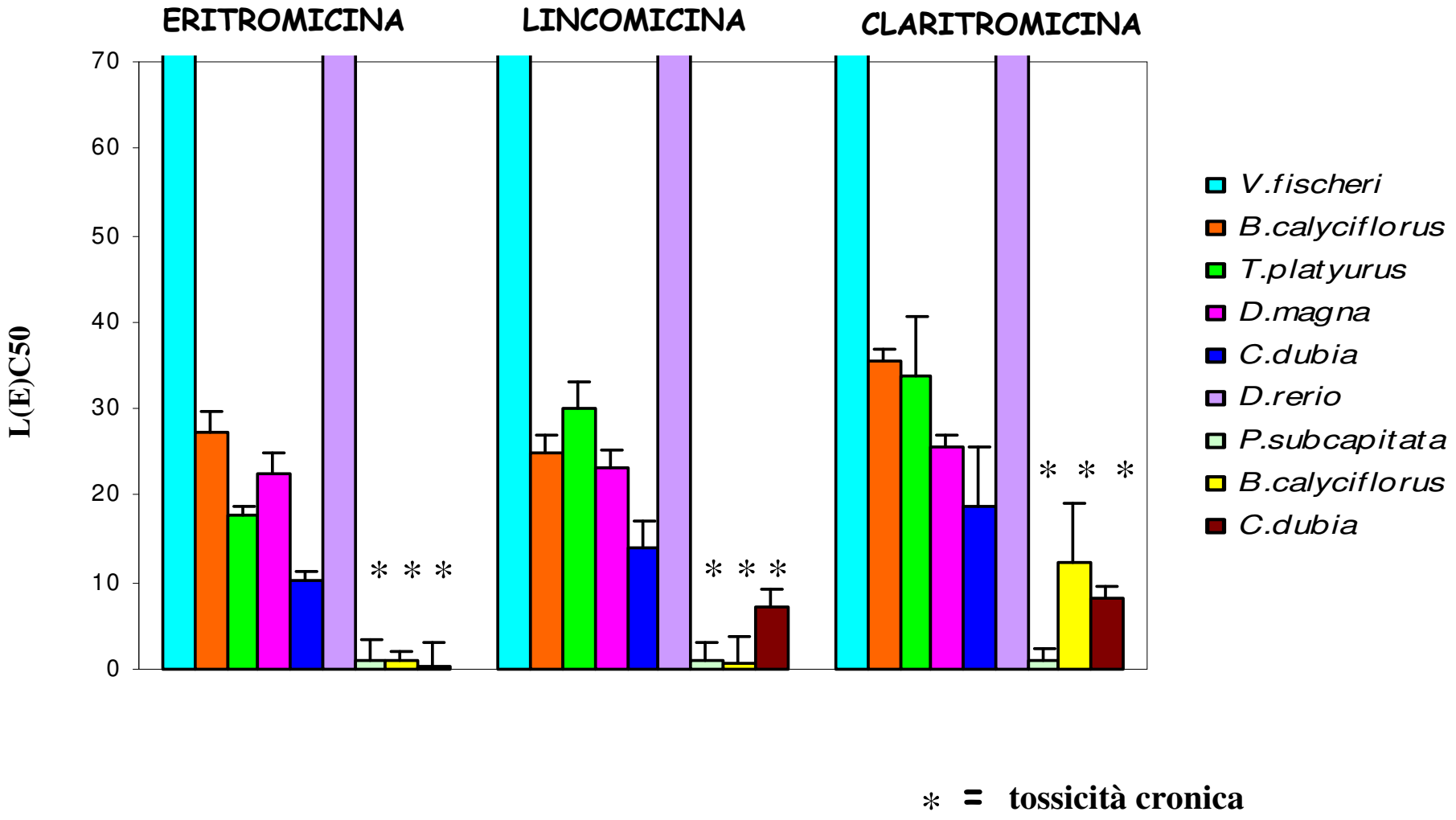
PNEC CRONICO

PNEC = valore cronico più basso EC50/1000 come per PNEC acuto

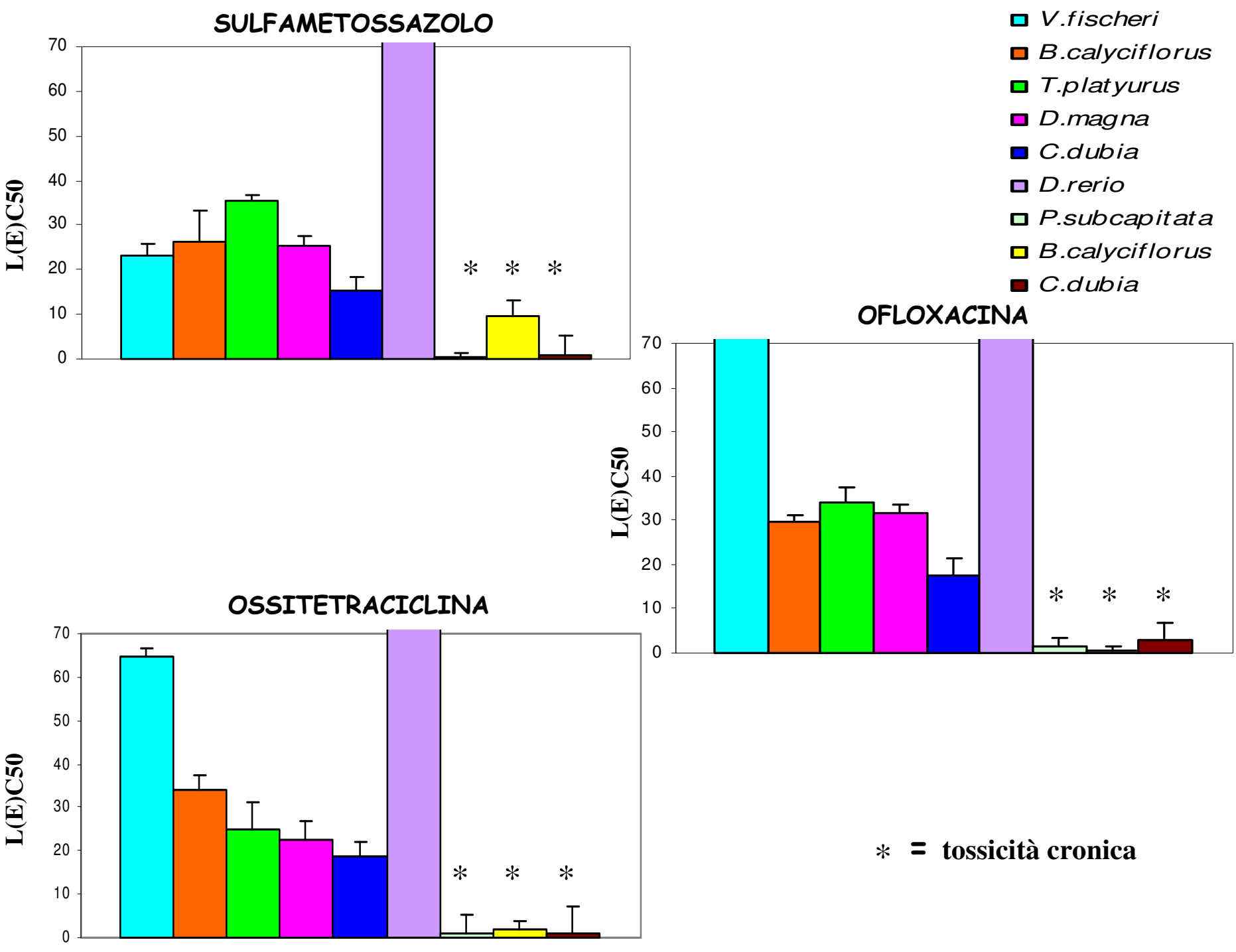
~~**PNEC = valore cronico più basso NOEC/AF AF=10, 50 or 100**~~



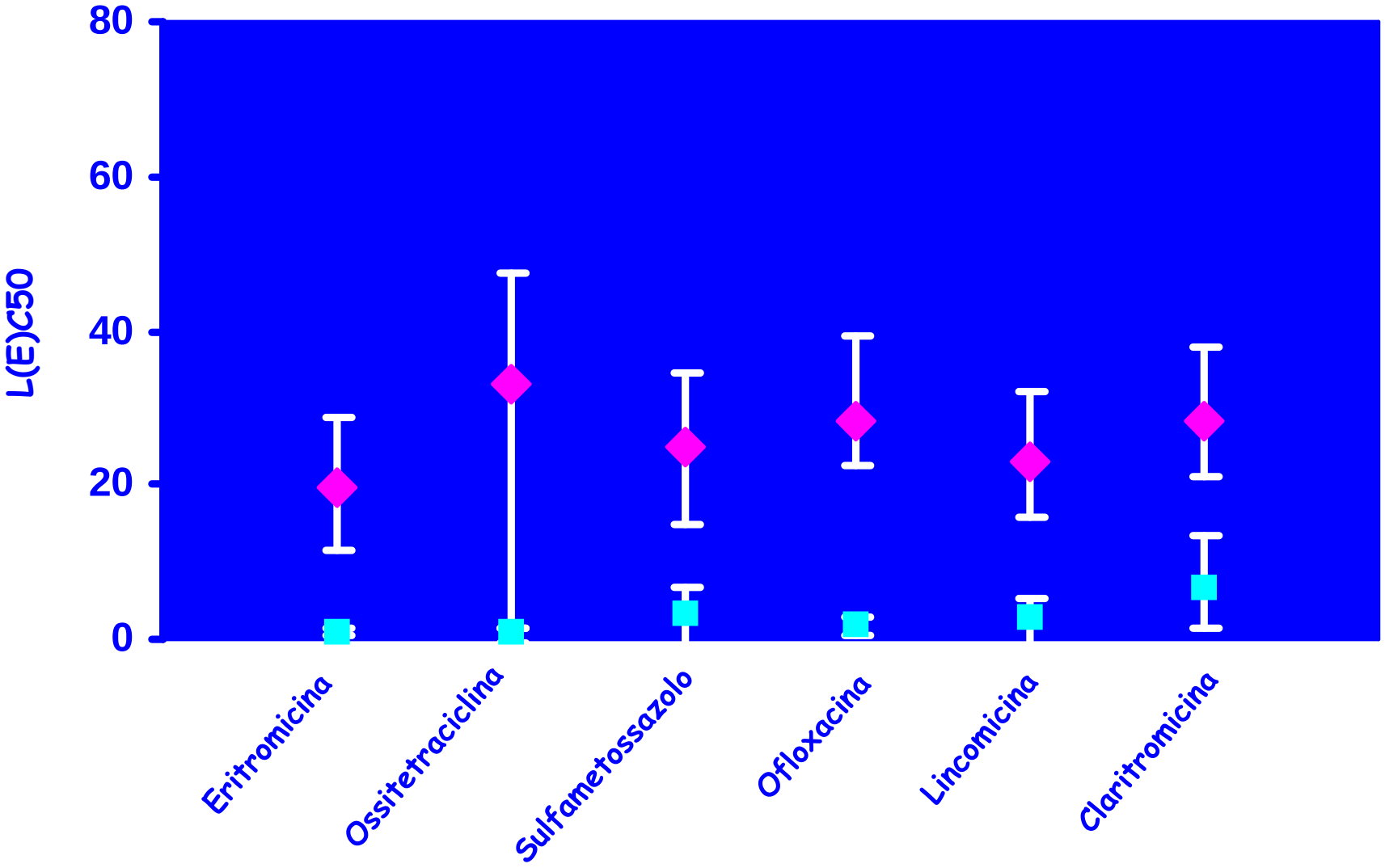
NOEC dipende dalla scelta delle concentrazioni e dalla loro scansione



* = **tossicità cronica**


SULFAMETOSSAZOLO
L(E)C50
70
60
50
40
30
20
10
0
* * *
V.fischeri
B.calyciflorus
T.platyurus
D.magna
C.dubia
D.rerio
P.subcapitata
B.calyciflorus
C.dubia
OFLOXACINA
L(E)C50
70
60
50
40
30
20
10
0
* * *
OSSITETRACICLINA
L(E)C50
70
60
50
40
30
20
10
0
* * *
* = tossicità cronica

CONFRONTO TRA VALORI MEDI ACUTI E CRONICI DI L(E)C50

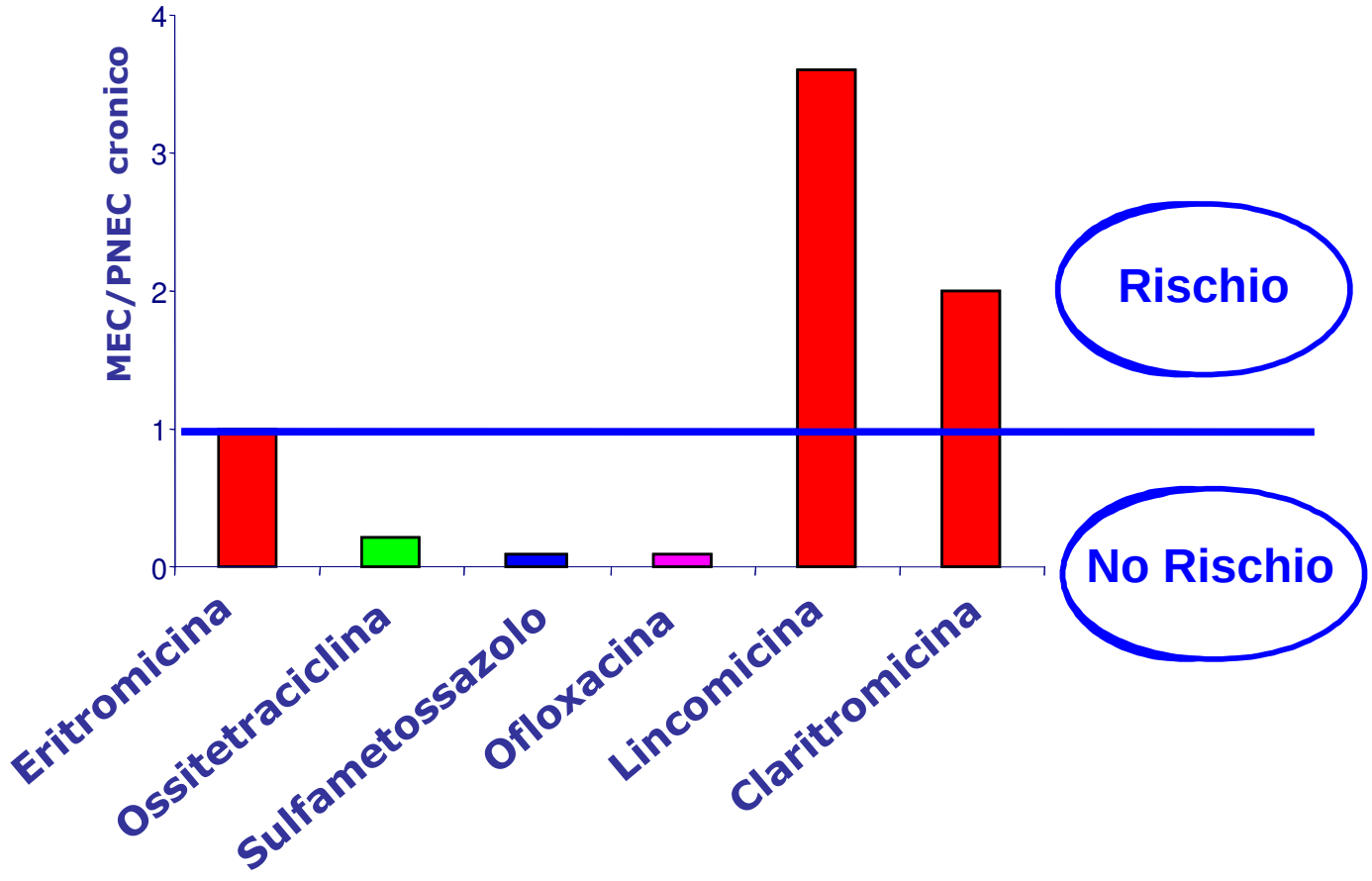


TEST DI AMES

	TA 98			TA 100		
Composti	**Intervallo di conc. mutagena (µg/L)**	**R^2**	**Massimo M.R.**	**R^2**	**Massimo M.R.**	**Intervallo di conc. mutagena (µg/L)**
Eritromicina	**-**	**0.44**	**0.84 ± 0.09**	**0.90**	**1.15 ± 0.36**	**-**
Ossitetraciclina	**-**	**0.96**	**10.4 ± 0.10**	**0.97**	**0.83 ± 0.43**	**-**
Sulfametossazolo	**6.25 - 100**	**0.96**	**14.53 ± 0.49**	**0.99**	**4.32 ± 0.55**	**50 - 100**
Ofloxacina	**0.315 - 2.5**	**0.93**	**72.32 ± 0.76**	**0.76**	**1.06 ± 0.33**	**-**
Lincomicina	**6.25 - 100**	**0.87**	**15.05 ± 2.11**	**0.95**	**4.66 ± 0.97**	**25 - 100**
Claritromicina	**-**	**0.99**	**1.71 ± 0.33**	**0.81**	**1.10 ± 0.26**	**-**

SOS CHROMOTEST

Composti	Intervallo di conc. mutagena (µg/L)	Massimo I.F.	R^2
Eritromicina	-	0.84 ± 0.06	0.98
Ossitetraciclina	-	0.97 ± 0.14	0.88
Sulfametossazolo	-	1.63 ± 0.18	0.10
Ofloxacina	6.25 - 25	78 ± 7.21	0.90
Lincomicina	-	1.46 ± 0.08	0.52
Claritromicina	-	1.20 ± 0.17	0.12


MEC/PNEC cronico
4
3
2
1
0
Rischio
No Rischio
Eritromicina
Ossitetraciclina
Sulfametossazolo
Ofloxacina
Lincomicina
Claritromicina

Toxicity intraspecies ratios
acute EC50/chronic EC50 (A/C ratio)

Compounds	*B. calyciflorus*	*C. dubia*
Atenolol	27	33
1 A	93.4	1372
Furosemide	> 40.16	35.7
1 Fu	> 116.50	133.9
Bezafibrate	138.4	583
1 B	> 48.6	51.8
2 B	14.9	12.3
Fenofibrate	45.1	> 131.6
1 F	0.2	45.9
Gemfibrozil	175.7	> 377.4
1 G	> 555.6	> 465.1

TOSSICITA' ACUTA

Compounds	*B. calyciflorus*	*C. dubia*
Bezafibrate	60.91 (54.03-68.66)	75.79 (60.13-81.01)
1B	NE 70	77.11 (65.41-84.09)
2B	109.32 (85.91-139.10)	90.57 (81.31-99.65)
Fenofibrate	64.97 (57.12-72.36)	NE 100
1Fe	46.29 (31.78-67.44)	42,24 (35.47-49.66)
Gemfibrozil	77.30 (59.12 – 101.08)	NE 200
Ranitidine	NE 100	NE 100
1R	2.10 (1.75-2.50)	0.59 (0.54-0.64)
2R	4.51 (3.52-5.95)	7.85 (6.43-9.32)
Erythromycin	27.53 (18.58-40.78)	10.23 (8.32-12.59)
Furosemide	NE 100	84.09 (70.11-91.01)
1Fu	NE 120	75.79 (64.31-79.12)
Tamoxifen	0.97 (0.82 - 1.14)	1.53 (1.26 – 1.85)

TOSSICITA' CRONICA

Compounds	*B. calyciflorus* (48 h)	*C. dubia* (7 gg)
Bezafibrate	0.44 (0.25-0.51)	0.13 (0.04-0.26)
1B	1.44 (1.08-1.91)	1.49 (0.74-2.65)
2B	7.36 (5.52-9.78)	7.35 (5.30-9.62)
Fenofibrate	1.44 (1.17-1.76)	0.76 (0.66-0.88)
1Fe	1.73 (1.42-2.10)	0.92 (0.80-0.98)
Gemfibrozil	0.44 (0.17 – 0.69)	0.53 (0.45-0.62)
Ranitidine	2.50 (1.99-3.24)	1.50 (1.13-1.94)
1R	0.25 (0.21-0.29)	0.007 (0.006-0.009)
2R	0.83 (0.62-1.11)	0.51 (0.39-0.65)
Erythromycin	0.94 (0.93-1.41)	0.22 (0.11-0.44)
Furosemide	2.493 (2.097-3.103)	2.354 (1.383-6.492)
1Fu	1.037 (0.761-1.385)	0.566 (0.275-3.018)
Tamoxifen	0.25 (0.10 - 0.38)	8.1×10^{-4} (2.2×10^{-4} – 1.4×10^{-3})

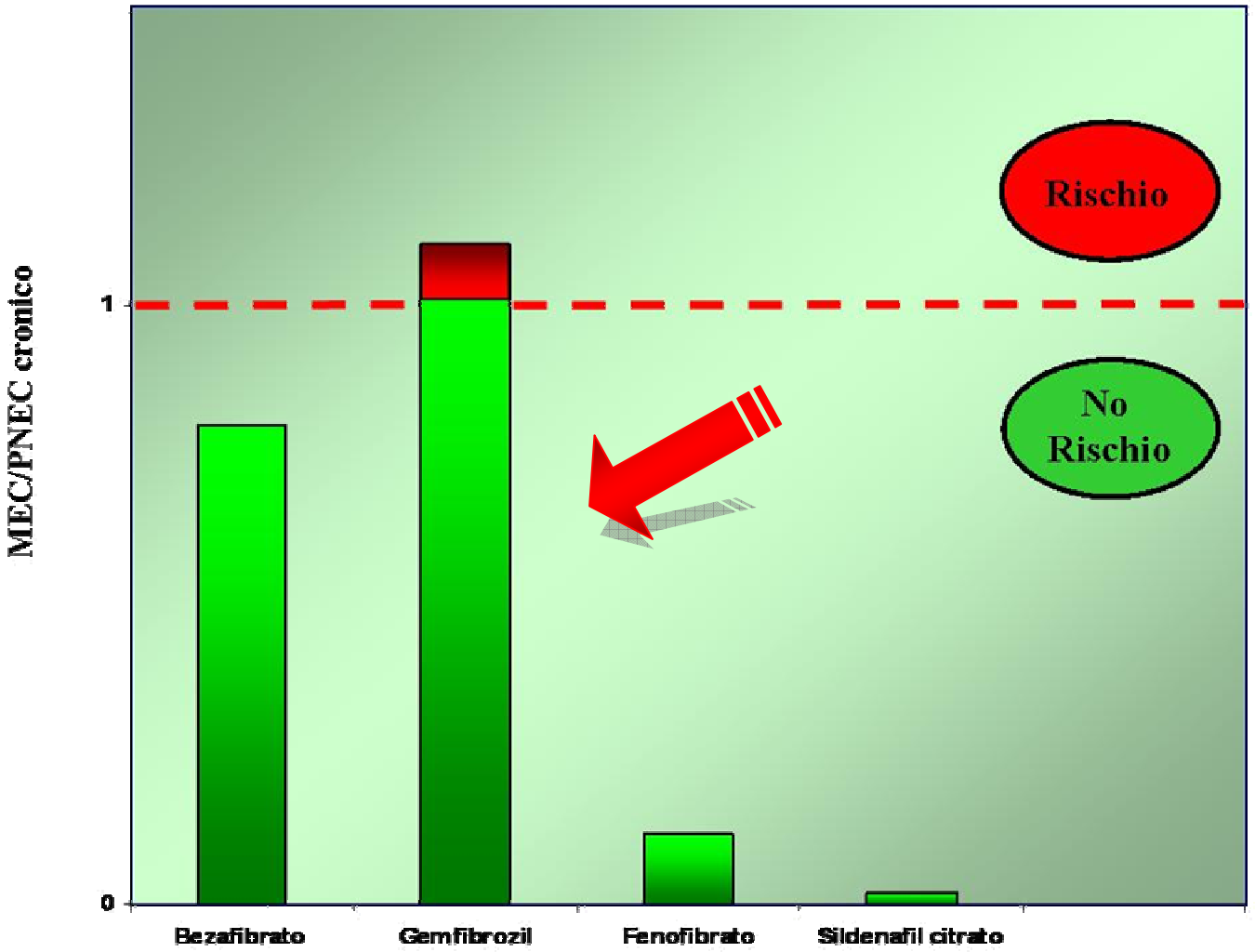
Damage score per la valutazione del danno

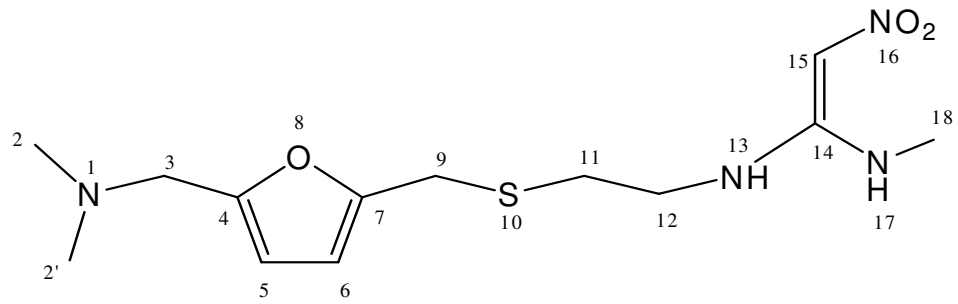
CAMPIONE	DAMAGE SCORE (0-200)
CONTROLLO NEGATIVO	11
CONTROLLO POSITIVO	19-135
GEMFIBROZIL	40-42
DERIVATO GEMFIBROZIL (1G)	89-84
BEZAFIBRATO	35-47
DERIVATO BEZAFIBATO (1B)	24-35
DERIVATO BEZAFIBATO (2B)	50-36
FENOFIBRATO	30-46
DERIVATO FENOFIBRATO (1F)	58-37
SILDENAFIL CITRATO	36-70

Concentrazioni saggiate comet = 0.05 – 2.00 µM

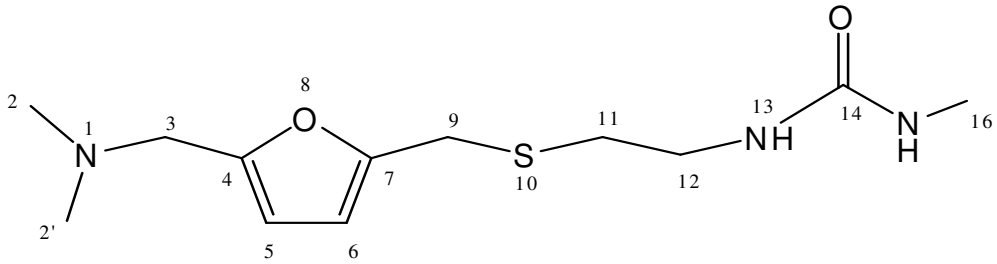
Schema riassuntivo genotossicità

CAMPIONI	Ames TA98	Ames TA100	SOS chromotest	Comet test
Gemfibrozil	-	-	-	-
Derivato Gemfibrozil (1G)	+	+	+	+
Bezafibrato	-	-	+	-
Derivato Bezafibrato (1B)	-	-	-	-
Derivato Bezafibrato (2B)	-	-	+	-
Fenofibrato	+	-	+	-
Derivato Fenofibrato (1F)	+	-	+	-
Sildenafil citrato	+	-	+	+

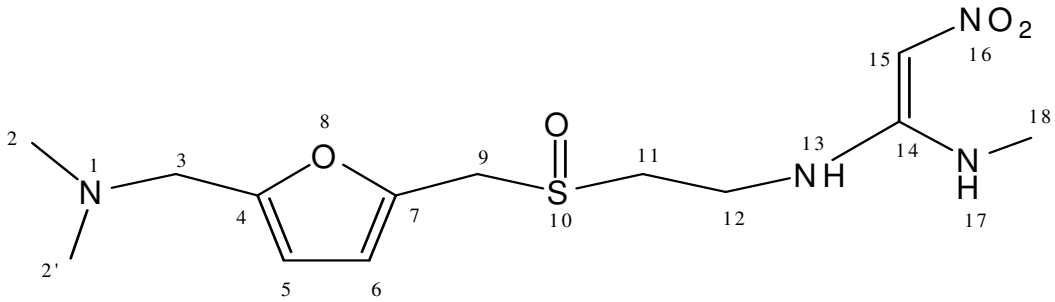

MEC/PNEC cronico
1
0
Rischio
No
Rischio
Bezafibrato
Gemfibrozil
Fenofibrato
Sildenafil citrato



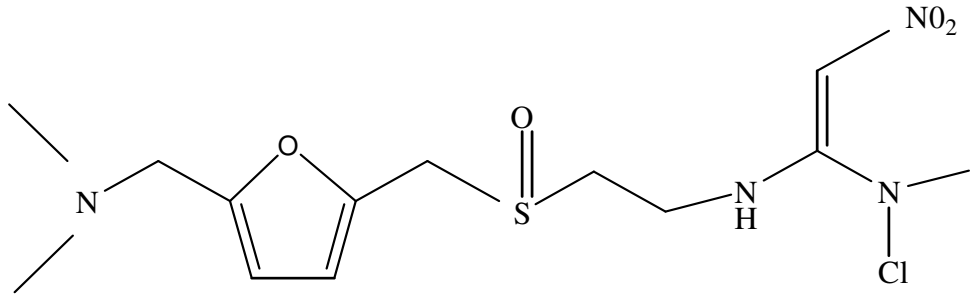
RANITIDINA



RA/44/2

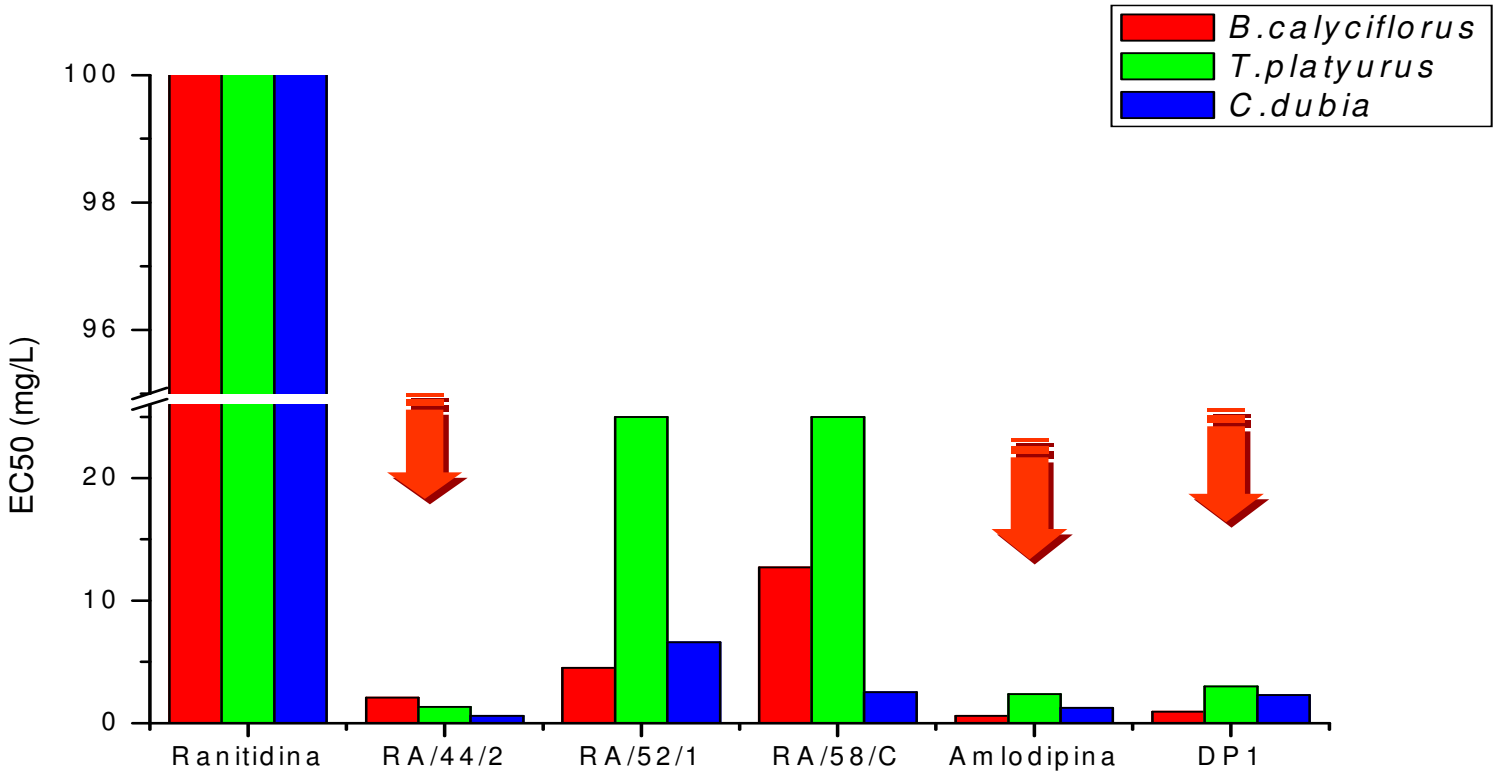


RA/52/1

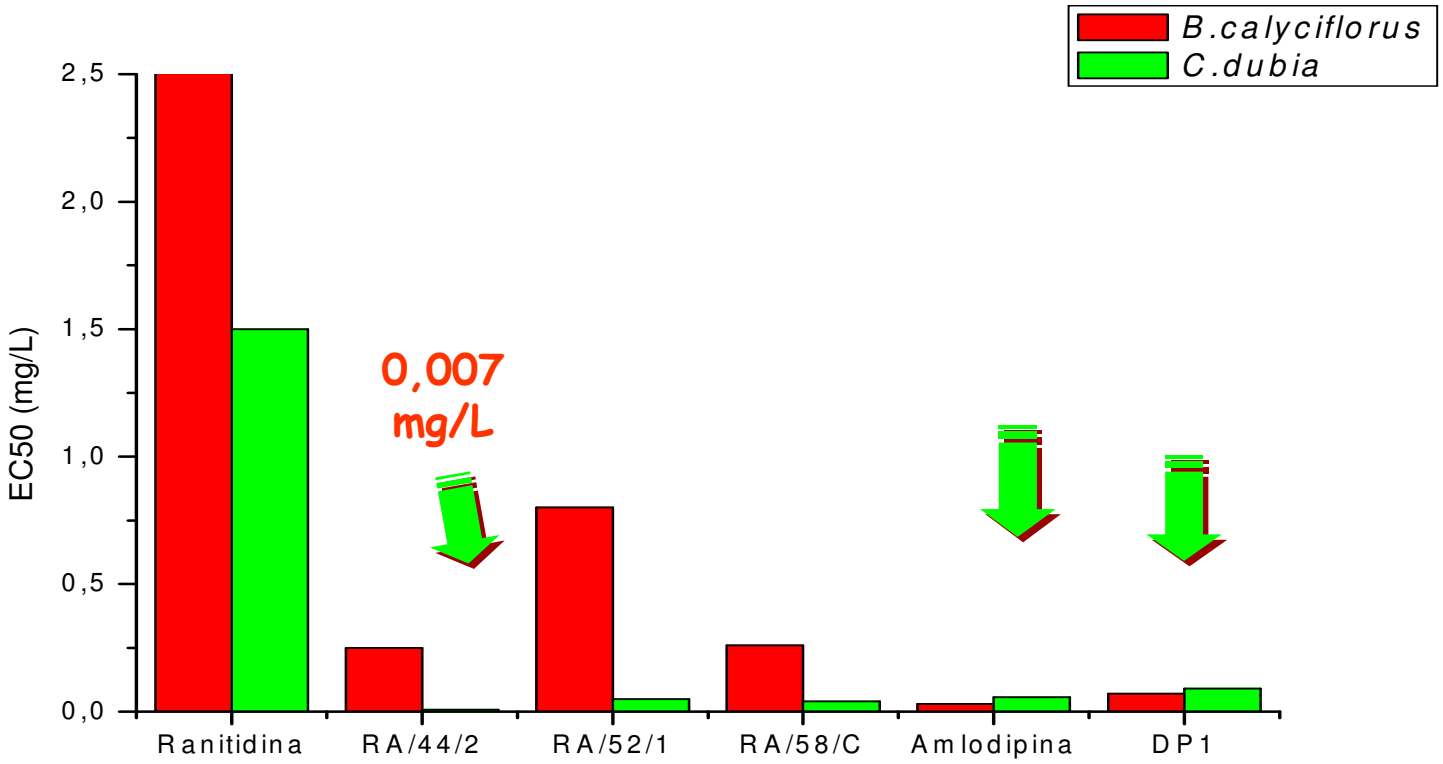


RA/58/C

TOSSICITA' ACUTA NEI CONFRONTI DEGLI ORGANISMI TESTATI



TOSSICITA' CRONICA NEI CONFRONTI DEGLI ORGANISMI TESTATI



$$R.M. = \frac{\text{n° revertenti/campione}}{\text{n° revertenti/controllo}}$$

Campione	TEST DI AMES								
	TA98			TA100			TA1535		
	Intervallo di conc. mutagena (mg/L)	Reverenti /µg	Max M.R.	Intervallo di conc. mutagena (mg/L)	Reverenti /µg	Max M.R.	Intervallo di conc. mutagena (mg/L)	Reverenti /µg	Max M.R.
Amlodipina	1-2	250	**6.2**	-	-	-	0.5-2	515	**6.8**
DP1	-	1060	1.9	-	14400	1.28	-	-	-
Ranitidina	50-100	28	**8.75**	-	15,93	1.3	-	-	-
RA/44/2	2.5-5	348	**3**	2.5-5	542	**3.2**	-	-	-
RA/52/1	0.3-0.6	1520	**2.7**	-	-	-	0.3-0.6	329,6	**3.2**
RA/58/C	-	1217	1.3	-	-	-	-	1512	1.7

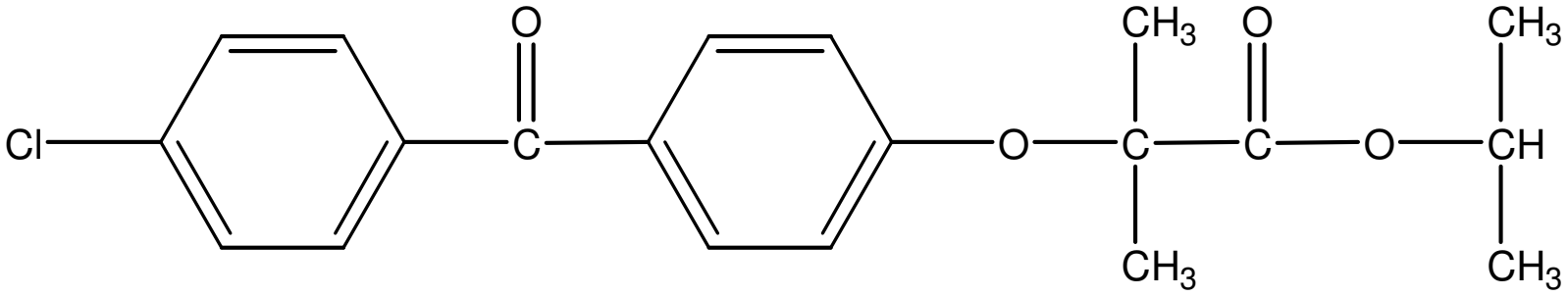
$$I.F. = \frac{\text{U.E. campione}}{\text{U.E. controllo}}$$

SOS CHROMOTEST			
Campioni	**Intervallo di conc. genotossica (mg/L)**	**I.F.**	**r**
Amlodipina	-	1.15	0.923
DP1	-	1.29	1.000
Ranitidina	12.5-25	**2.29**	0.943
RA/44/2	0.31-5	**3.55**	0.901
RA/52/1	0.31-5	**3.57**	0.839
RA/58/C	2.5-5	**2.07**	1.000

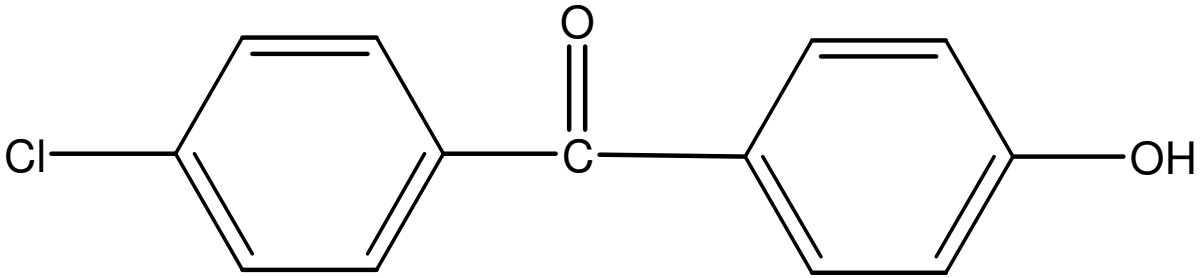
RIASSUMENDO.....

Campioni	Test di Ames			Sos Chromotest
	TA98	TA100	TA1535	
Amlodipina	+		+	-
DP1	-	-		-
Ranitidina	+	-		+
RA/44/2	+	+		+
RA/52/1	+		+	+
RA/58/C	-		-	+

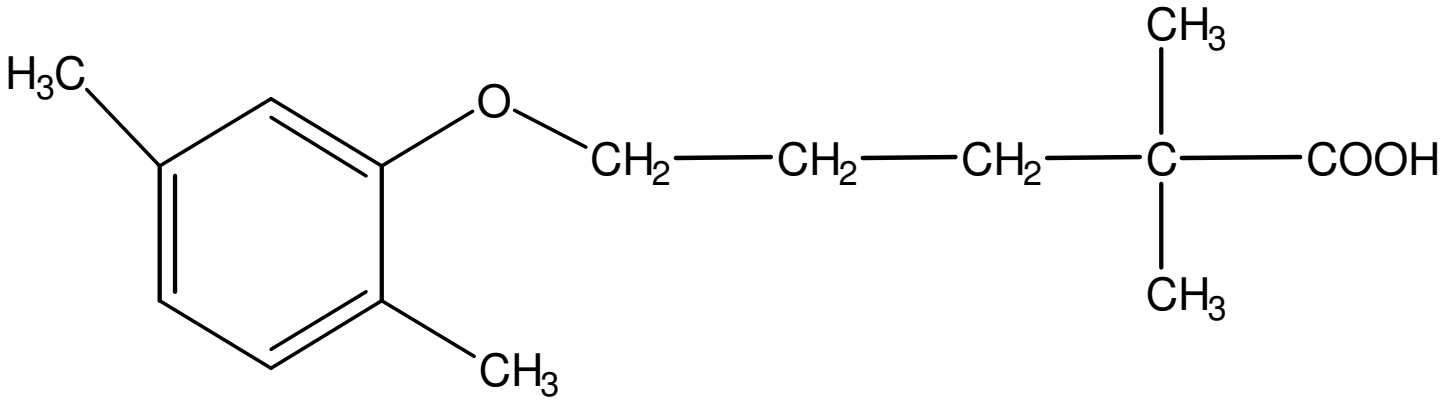
FENOFIBRATO



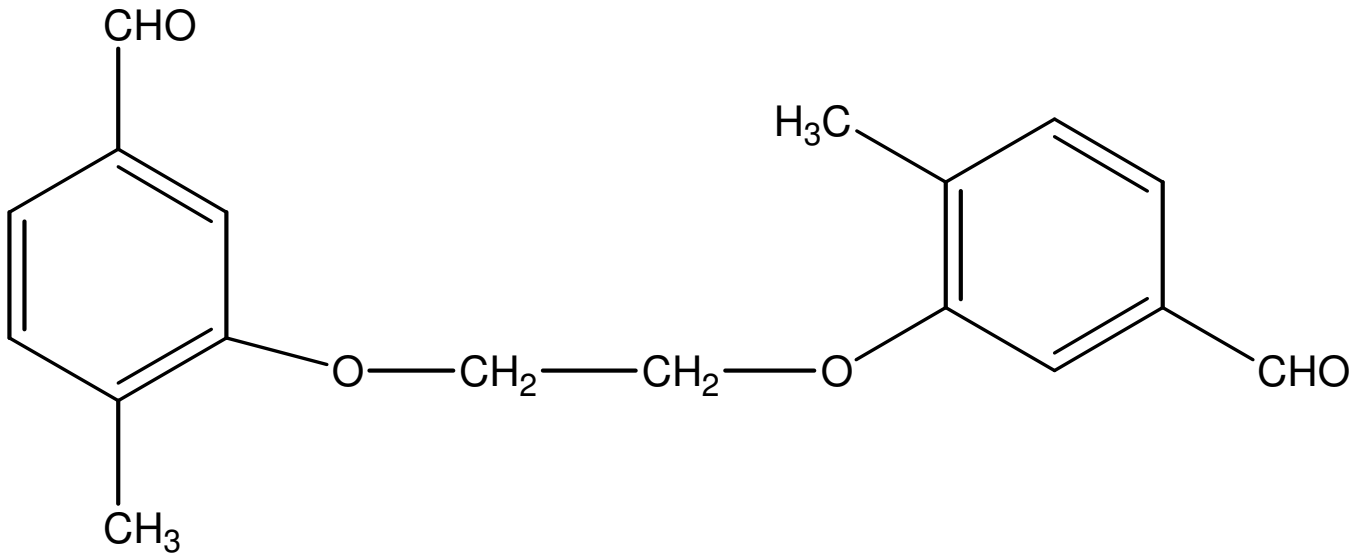
Derivato Fenofibrato (1F)



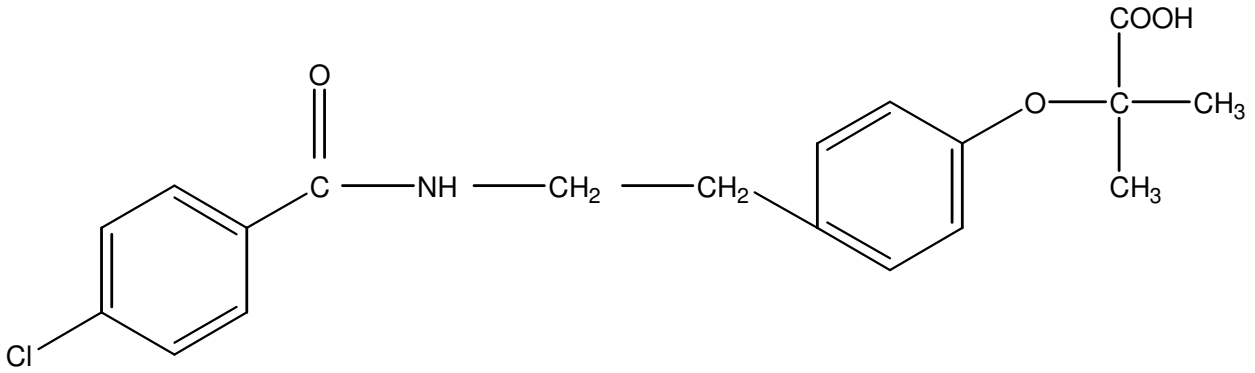
GEMFIBROZIL



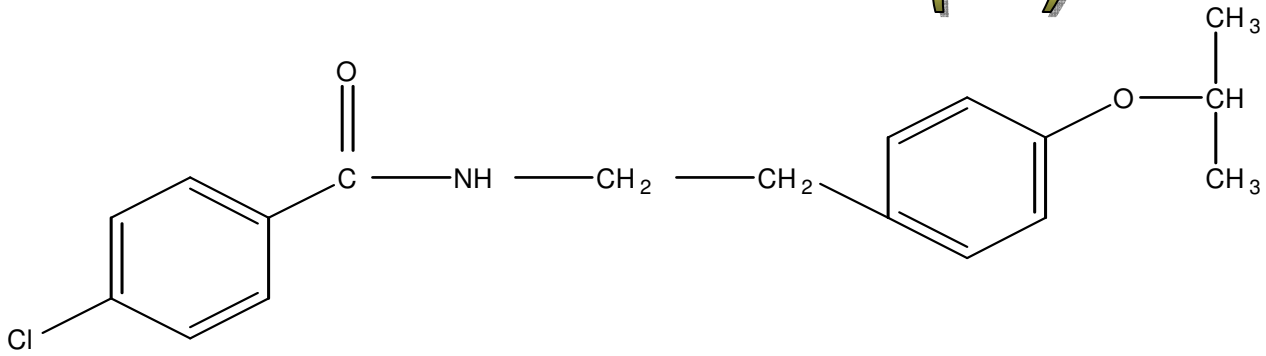
Derivato Gemfibrozil (1G)



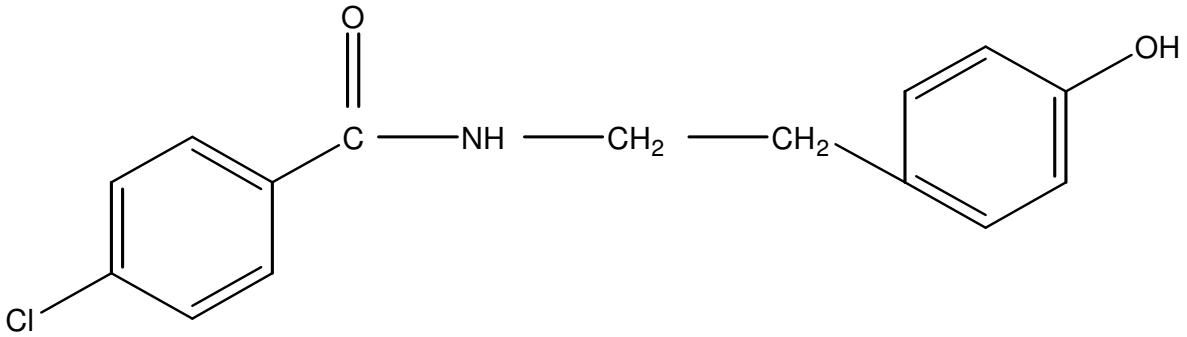
BEZAFIBRATO



Derivato Bezafibrato (1B)



Derivato Bezafibrato (2B)



Damage score per la valutazione del danno

CAMPIONE	DAMAGE SCORE (0-200)
CONTROLLO NEGATIVO	11
CONTROLLO POSITIVO	19-135
GEMFIBROZIL	40-42
DERIVATO GEMFIBROZIL (1G)	89-84
BEZAFIBRATO	35-47
DERIVATO BEZAFIBATO (1B)	24-35
DERIVATO BEZAFIBATO (2B)	50-36
FENOFIBRATO	30-46
DERIVATO FENOFIBRATO (1F)	58-37
SILDENAFIL CITRATO	36-70

Schema riassuntivo genotossicità

CAMPIONI	Ames TA98	Ames TA100	SOS chromotest	Comet test
Gemfibrozil	-	-	-	-
Derivato Gemfibrozil (1G)	+	+	+	+
Bezafibrato	-	-	+	-
Derivato Bezafibrato (1B)	-	-	-	-
Derivato Bezafibrato (2B)	-	-	+	-
Fenofibrato	+	-	+	-
Derivato Fenofibrato (1F)	+	-	+	-
Sildenafil citrato	+	-	+	+

TEST DI AMES

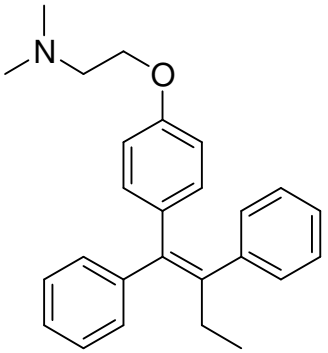
	TA98			TA100		
Composti	Intervallo di conc. mutagena (µg/mL)	R^2	Massimo R.M.	Intervallo di conc. mutagena (µg/mL)	R^2	Massimo R.M.
Bezafibrato	-	0,90	1,58 ± 0,16	-	0,79	1,10 ± 0,46
1B	-	0,85	1,64 ± 2,5	-	0,95	1,79 ± 0,82
2B	-	0,38	1,38 ± 0,5	-	0,78	0,34 ± 0,26
Fenofibrato	0,625-10	0,96	10,70 ± 2,7	-	0,96	0,93 ± 0,31
1F	0,625-10	0,99	11,13 ± 0,6	-	0,77	0,96 ± 0,52
Gemfibrozil	-	0,77	1,46 ± 0,12	-	0,64	1,17 ± 1,1
1G	0,625-10	0,96	121 ± 0,6	0,625-10	0,86	21,90 ± 1,4
Sildenafil citrato	0,625-10	0,99	4,49 ± 0,5	-	0,88	1,37 ± 1,5

Rapporti di tossicità intraspecie

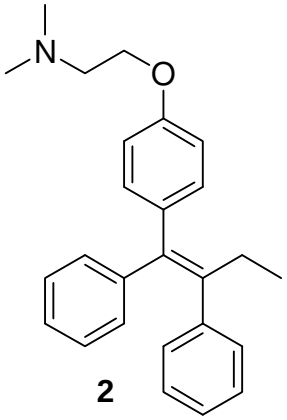
EC50 acuta/ EC50 cronica

Composti	*B. calyciflorus*	*C. dubia*
Bezafibrato	138.4	583
1B	> 48.6	51.8
2B	14.9	12.3
Fenofibrato	45.1	> 131.6
1F	0.2	45.9
Gemfibrozil	175.7	> 377.4
1G	> 555.6	> 465.1
Sildenafil citrato	17.2	8.97

TAMOXIFENE E FOTODERIVATI



Tamoxifene (1)



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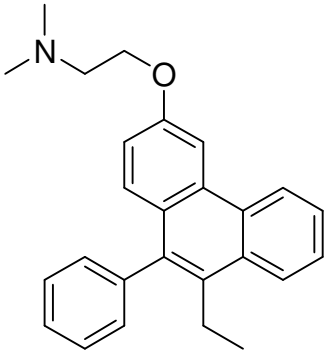

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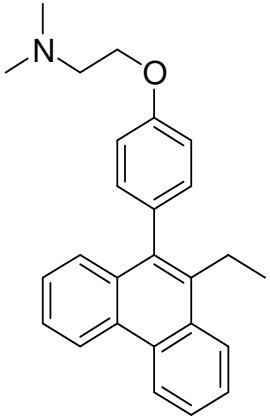

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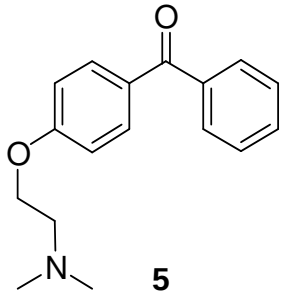
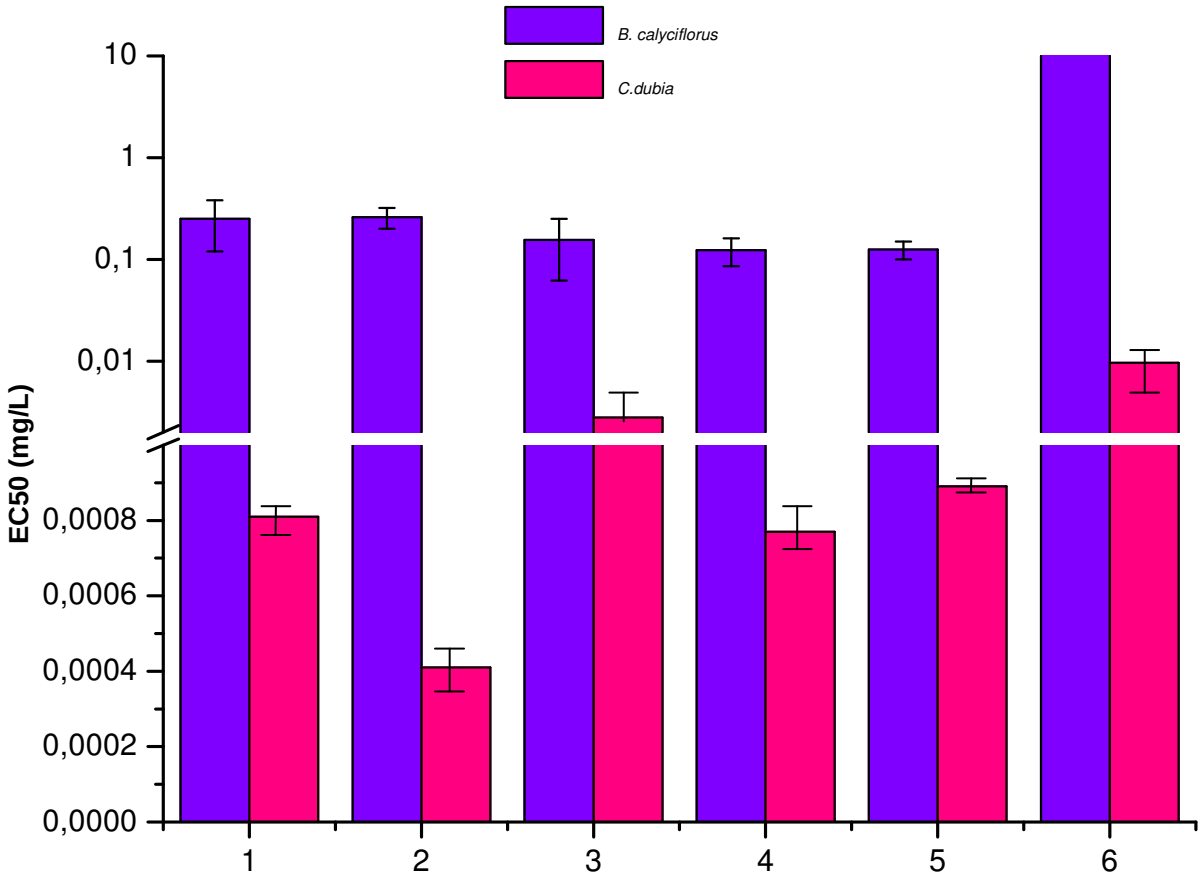


5

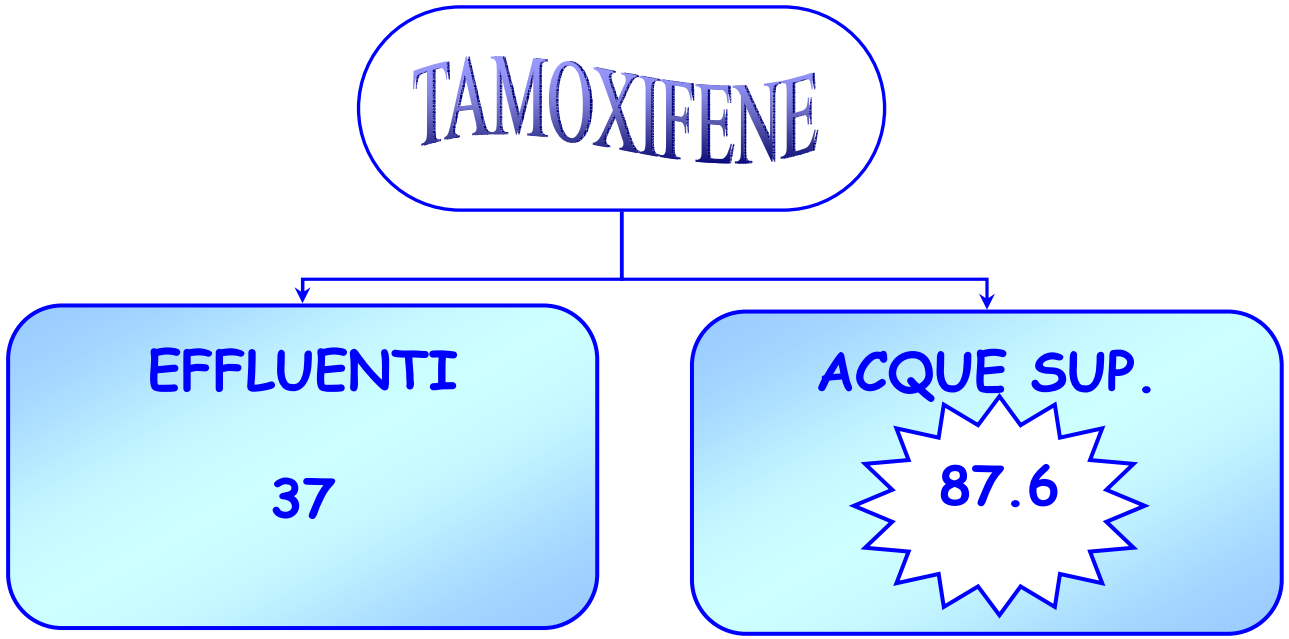
Risultati di tossicità cronica nei confronti dei diversi organismi saggiati



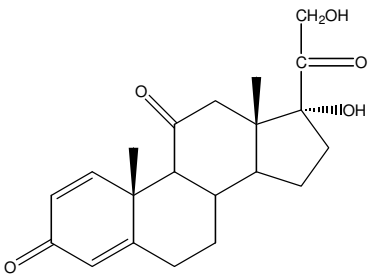
Rapporti di tossicità intraspecie
EC50 acuta/ EC50 cronica

COMPOSTI	B. CALYCIFLORUS
1	3.8
2	4.11
3	6.09
4	8.61
5	10.48
Miscela irradiata	1.14

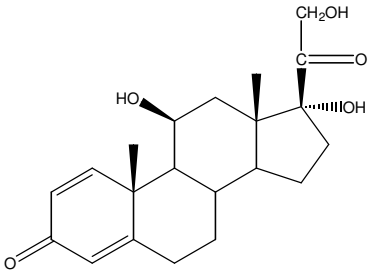
Rischio ambientale cronico



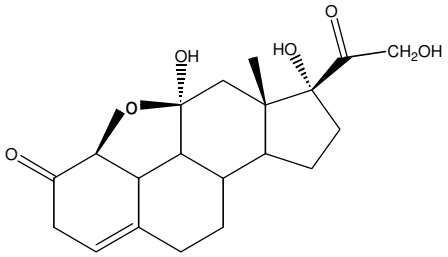
Il Prednisone e i suoi derivati



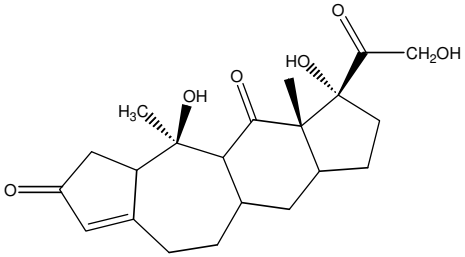
Prednisone (**1**)



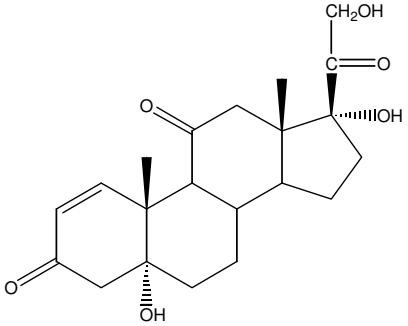
Prednisolone (**2**)



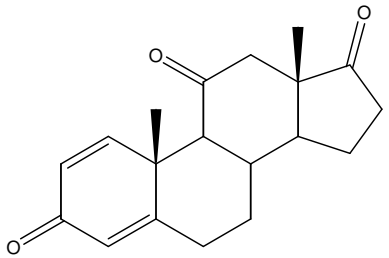
(**3**)



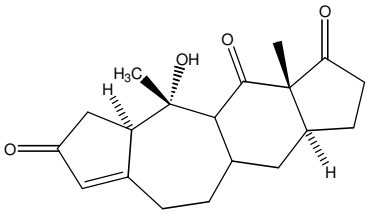
(**4**)



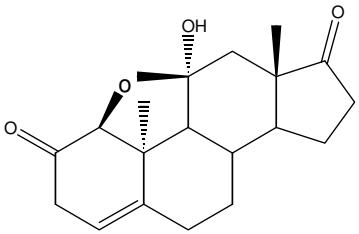
(**5**)



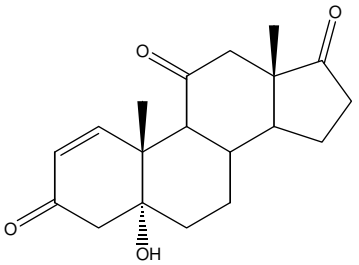
Δ^1–Adrenosterone (**6**)



(**7**)



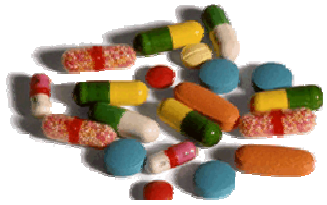
(**8**)



(**9**)

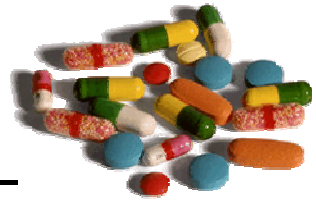
Valori di L(E)C50 espressi in μM relativi ai saggi di tossicità acuta

Composto	*Daphnia magna* 24h	*Daphnia magna* 48h	*Thamnocephalus platyurus*	*Brachionus calyciflorus*
1	NE 279	30% di effetto a 279	23% di effetto a 447	152.2 *(107-221)*
2	NE 236	45% di effetto a 236	23% di effetto a 389	61.91 *(21.36-179.3)*
3	NE 106	35% di effetto a 106	30% di effetto a 133	129.3 (106.1-157.7)
4	220 *(198-245)*	84.49 *(55.8-127.7)*	141 *(115-182)*	311 *(260.1-330.5)*
5	94.93 *(59.8-150.6)*	53.3 *(34.4-90.34)*	36% di effetto a 212.3	43% di effetto a 159.4
6	82.9 *(64.8-106)*	44.9 *(58.7-76.7)*	20.5 *(19.3-21.7)*	100.3 *(75.4-133.3)*
7	NE 253	NE 253	17% di effetto a 316	10.56 *(6.8-14.4)*
8	102.7 *(95.9-110)*	84.7 *(73.2-98)*	136.80 *(110-170)*	176.8 *(172.6-181.2)*
9	35% di effetto a 158	108.1 *(101-115.7)*	26% di effetto a 158.2	91.04 *(61.9-146.13)*



Valori di EC50 espressi in µM relativi ai saggi di tossicità cronica

Composto	*Ceriodaphnia dubia* (test a 7 gg)	LOEC	NOEC	*Selenastrum capricornutum* (test a 3gg)
1	> 5.58	-	-	85.5 (69.3-103.5)
2	0.64 (0.36-1.3)	0.014	0.0038	488 (407-634)
3	0.77 (0.61-1.03)	0.035	0.026	342 (268-447)
4	0.42 (0.32-0.56)	0.047	0.026	NE 425
5	0.45 (0.34-0.61)	0.030	0.022	195 (154-252)
6	0.43 (0.30-0.60)	0.023	0.015	75 (72-79)
7	0.056 (0.032-0.085)	0.00066	0.00041	850.2
8	0.056 (0.035-0.082)	0.0007	0.0004	93.6 (84.1-104)
9	0.11 (0.06-0.16)	0.0025	0.0016	55 (39.2-77)



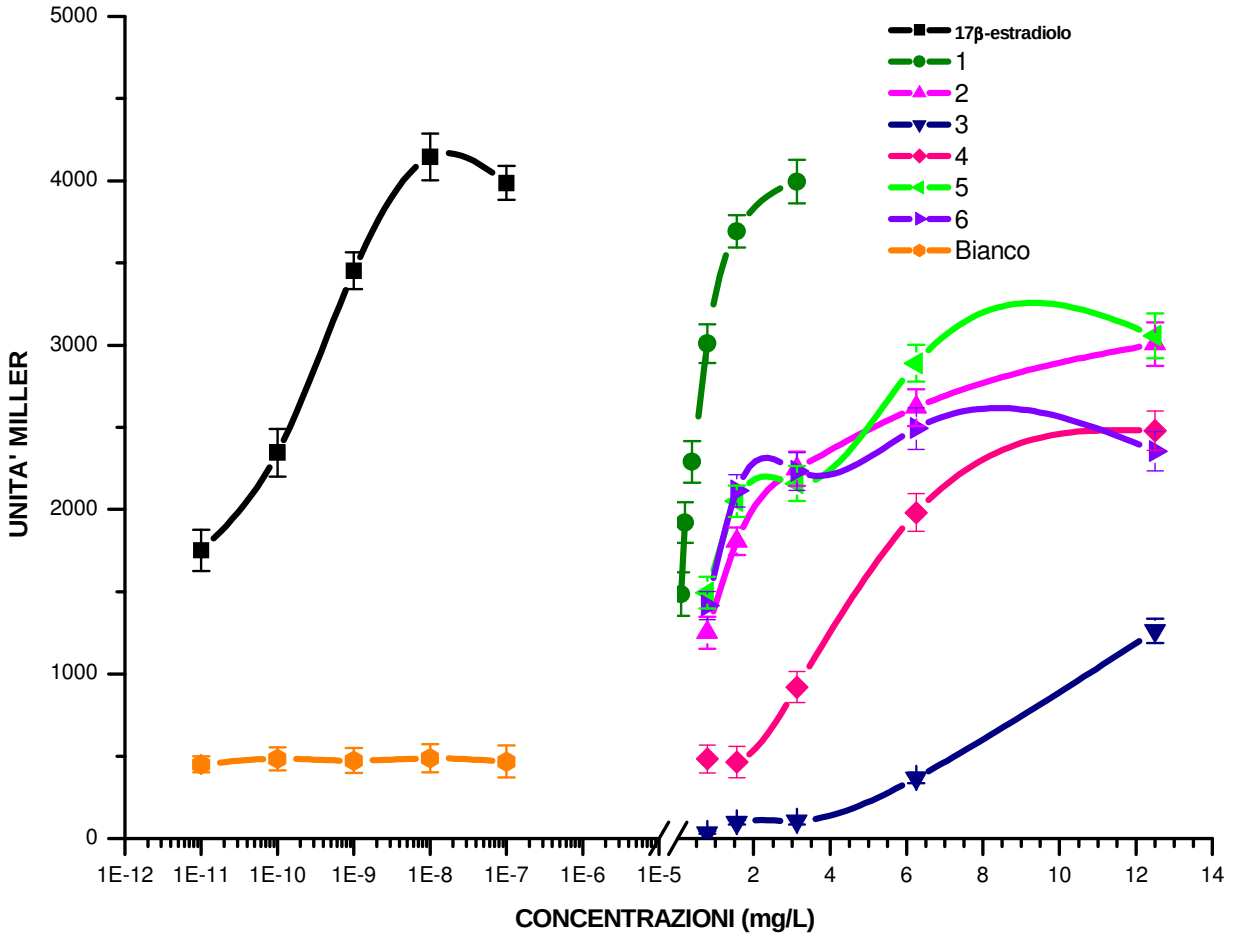
NE= nessun effetto fino a
ND=non determinato

Valori di L(E)C50 espressi in µM, relativi ai saggi di tossicità cronica. I limiti di confidenza al 95% sono riportati in parentesi.

INTERFERENTI ENDOCRINI

Secondo la definizione adottata dall'Unione Europea "un interferente endocrino è una sostanza esogena, o una miscela, che altera la funzionalità del sistema endocrino, causando effetti avversi sulla salute di un organismo o sulla sua progenie".

Curve dose/risposta del 17β-estradiolo, del tamoxifene e dei suoi fotoderivati



SEVENTH FRAMEWORK PROGRAMME

THEME [ENV.2010.1.2.2-2]
[Human health and environmental effects of exposure to pharmaceuticals released into the environment]

Grant agreement for: Collaborative project

Annex I - "Description of Work"

Project acronym: CytoThreat

Project full title: " Fate and effects of cytostatic pharmaceuticals in the environment and the identification of biomarkers for and improved risk assessment on environmental exposure "

Grant agreement no: 265264

Date of preparation of Annex I (latest version): 2010-08-23

Date of last change: 2010-08-10

Date of approval of Annex I by Commission:

FATE AND EFFECTS OF CYTOSTATIC PHARMACEUTICALS IN THE ENVIRONMENT AND IDENTIFICATION OF BIOMARKERS FOR AN IMPROVED RISK ASSESSMENT ON ENVIRONMENTAL EXPOSURE (CytoThreat)

Metka Filipič[1], Ester Heath [2], Marina Isidori [3], Siegfried Knasmuller [4], AkoHorvat s[5], Verica Garaj Vrhovac [6], Goran Gačić [7]

[1]National Institute of Biology, Ljubljana, Slovenia, metka.filipic@nib.si; [2]Jozef Stefan Institute, Ljubljana, Slovenia, [3]Second University of Naples, Caserta, Italy; [4]Medical University of Vienna, Vienna, Austria; [5]Szent István University, Godolo, Hungary; [6]Institute for Medical Research and Occupational Health, Zagreb, Croatia; [7]Institute for Multidisciplinary Research (IMSI), Belgrade, Serbia

The FP7 project CytoThreat addresses the needs of the European society for assessing the risks associated with the release of pharmaceuticals into environment. It focuses on cytostatics that are due to their genotoxic mechanism of action, highly hazardous compounds, but the data that would allow for adequate risk assessment is insufficient. The research will address occurrence, distribution and fate of selected cytostatics in aquatic matrices, and their chronic toxicity and genotoxicity to aquatic organisms. A combination of state-of-the art analytical chemistry, *in vivo* and *in vitro* systems, cell biology, and "OMICS" technologies will be applied. The *in vivo* studies with zebrafish models in combination with transcripotmic and bioinformatics are aimed to identify linkages between the genomic profiles, exposure conditions and adverse outcomes, to identify molecular biomarkers of exposure and effects of specific groups of cytostatics that will serve as diagnostic markers for these types of pharmaceutical exposure, and to predict synergistic effects at combined exposures. Comparative *in vitro* genotoxicity and transcriptomic studies with zebrafish and human cells are aimed to provide basis for more reliable extrapolation of toxicological data from zebrafish models to humans. CytoThreat is expected to generate new knowledge on potential environmental and health risk of cytostatics in the environment, providing objective arguments for recommendations and

FP7 GRANT AGREEMENT

ANNEX IV - FORM A - ACCESSION OF BENEFICIARIES TO THE GRANT AGREEMENT

SECONDA UNIVERSITÀ DEGLI STUDI DI NAPOLI, represented for the purpose hereof by Pietro Monaco, Director of the Department of Life Sciences, or his authorised representative, established in VIALE BENEDUCE 10, CASERTA, 81100, Italy acting as its legal authorised representative, hereby consents to become a beneficiary ("beneficiary no. 5") to grant agreement N° 265264 (relating to project "Fate and effects of cytostatic pharmaceuticals in the environment and the identification of biomarkers for and improved risk assessment on environmental exposure") concluded between the European Commission and NACIONALNI INSTITUT ZA BIOLOGIJO established in VECNA POT - 111, LJUBLJANA, 1000, Slovenia and accepts in accordance with the provisions of the aforementioned grant agreement all the rights and obligations of a beneficiary.

Done in 3 copies, of which one shall be kept by the coordinator and one by SECONDA UNIVERSITÀ DEGLI STUDI DI NAPOLI, the third being sent to the Commission by the coordinator in accordance with Articles 1.1 and 1.2 and Article 8 of the grant agreement.

SECONDA UNIVERSITÀ DEGLI STUDI DI NAPOLI	NACIONALNI INSTITUT ZA BIOLOGIJO
.....	.....
Name of legal representative(s)	Name of legal representative(s)
.....	.....
Signature of legal representative(s)	Signature of legal representative(s)
.....	.....
Date	Date
.....	.....
Stamp of the organisation	Stamp of the organisation