Biomarcadores moleculares para la evaluación de la respuesta tóxica en invertebrados modelo y no modelo

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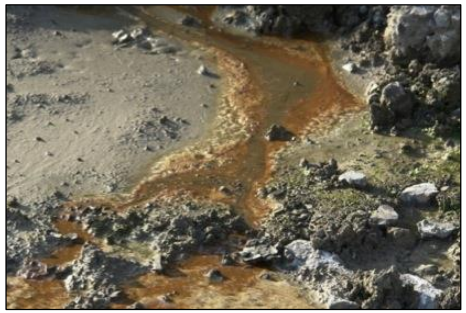
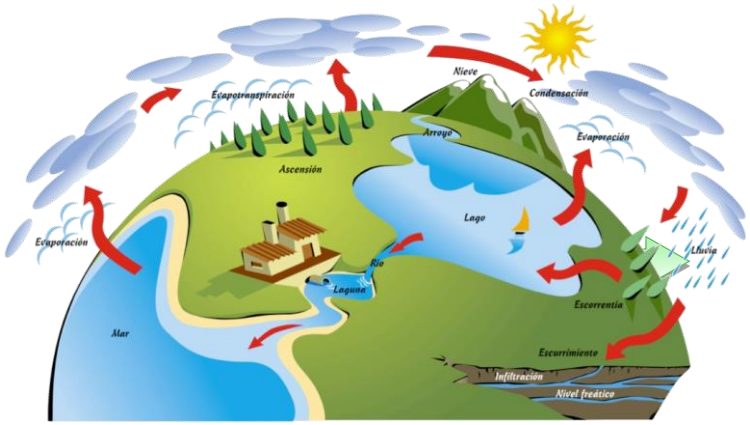
II Jornadas de Toxicología Ambiental y Ecotoxicología
ToxEcotox 2019
31 Ene 2019 Madrid

ENVIRONMENTAL POLLUTION



The pollutants are integrated in a cycle that involves:

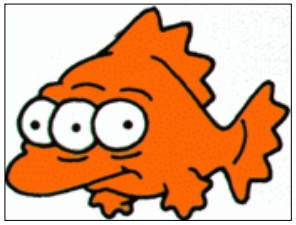


- Water
- Sediments
- Organisms

CHEMICAL APPROACH ⟷ **BIOLOGICAL APPROACH**

POLLUTANTS

EFFECTS



ENVIRONMENTAL POLLUTION

Ubiquitous substances that can be found in **everyday objects.**

Phtalates
(plasticizers)

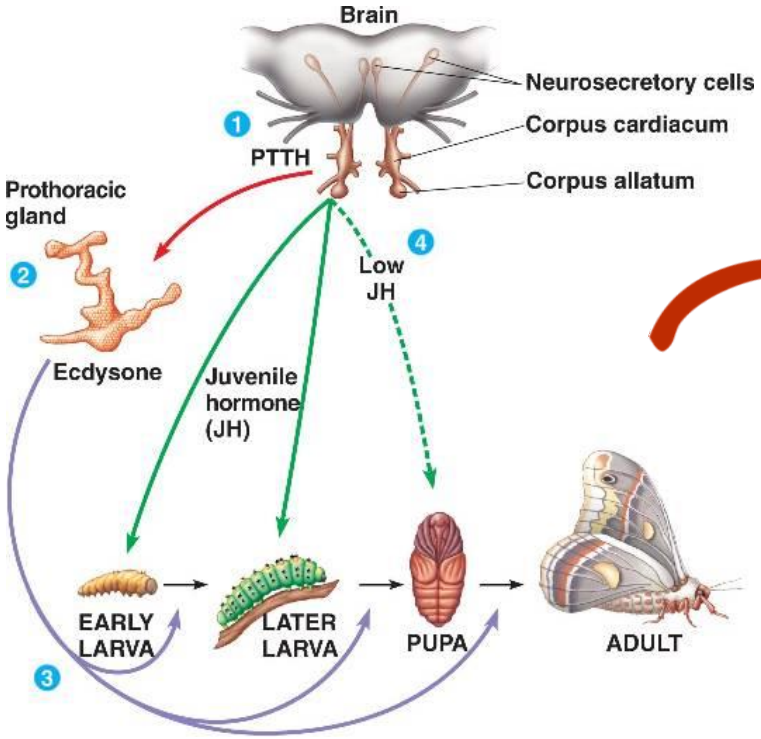
Bisphenol A
(plasticizer)

Perfluorooctanoic acid
(surfactant)

DDT
(insecticide)

WHAT HAPPEN WITH INSECTS?

Insects represent more than 80% of all described animal species. They play **important and diverse ecological roles** in many environments. Since insects hormones regulate many behavioral and physiological processes, they are **very susceptible to ECDs** effects.



Embryogenesis
Metamorphosis
Reproduction
Growth
Molting
Behavior

MODEL SPECIES

Among invertebrates, **insects** are **excellent models** for studying endocrine systems and for **testing toxic chemicals**.

Tribolium castaneum

Anopheles gambiae

Drosophila melanogaster

Bombyx mori

Apis mellifera

Acyrthosiphon pisum

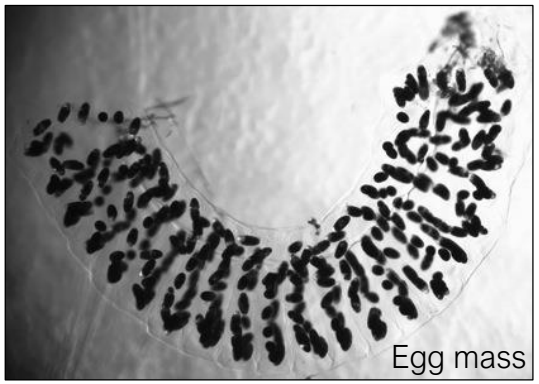
Chironomus riparius

Chironomus riparius

- *C. riparius* is a sediment species widely used in Ecotoxicology, with four OECD **standardized methods**.
- Traditional ecotoxicity endpoints in *C. riparius* include acute, **developmental and life-cycle effects**.
- In recent years, **molecular biomarkers** have been successfully used to shed light on the mechanisms of action of pollutants.

WHY?

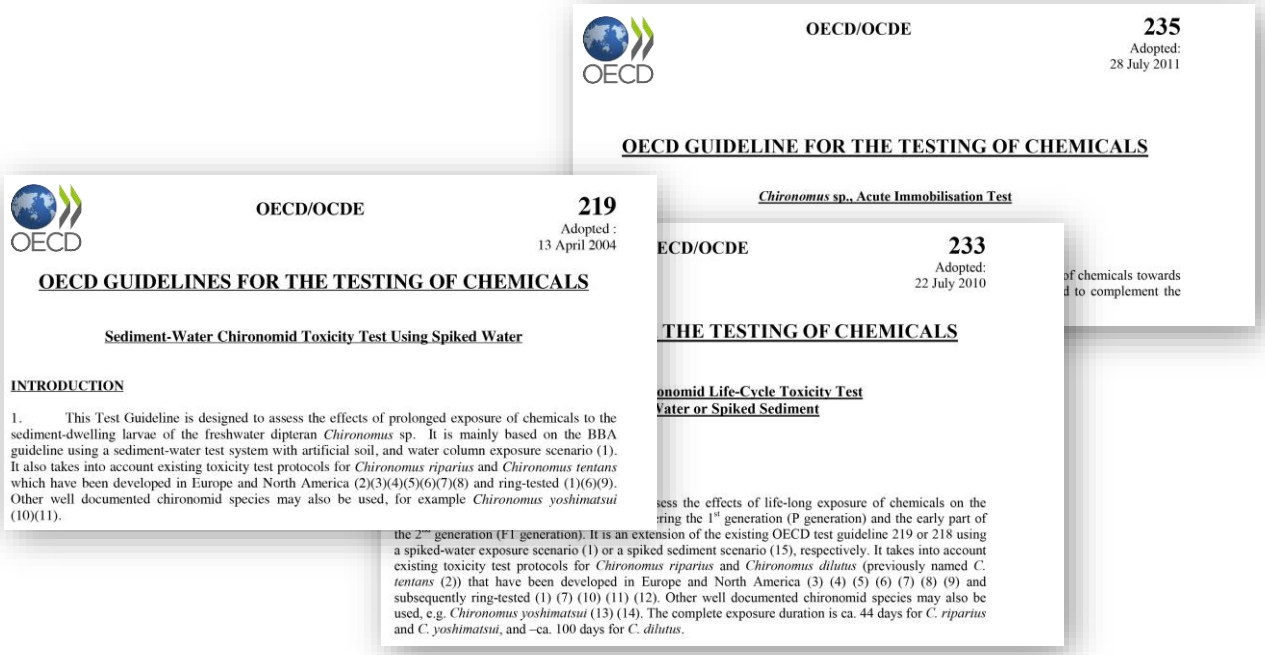
- *Wide distribution (ubiquitous)*
- *Associated with aquatic environments*
- *Essential for the balance of food chains*
- *Model species in ecotoxicity studies*
- *Four larval stages*
- *Associated with sediments*


Egg mass


4th instar larvae


Adult

Chironomus riparius



OECD/OCDE 235
Adopted: 28 July 2011

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Chironomus sp., Acute Immobilisation Test

OECD/OCDE 219
Adopted : 13 April 2004

OECD GUIDELINES FOR THE TESTING OF CHEMICALS

Sediment-Water Chironomid Toxicity Test Using Spiked Water

INTRODUCTION

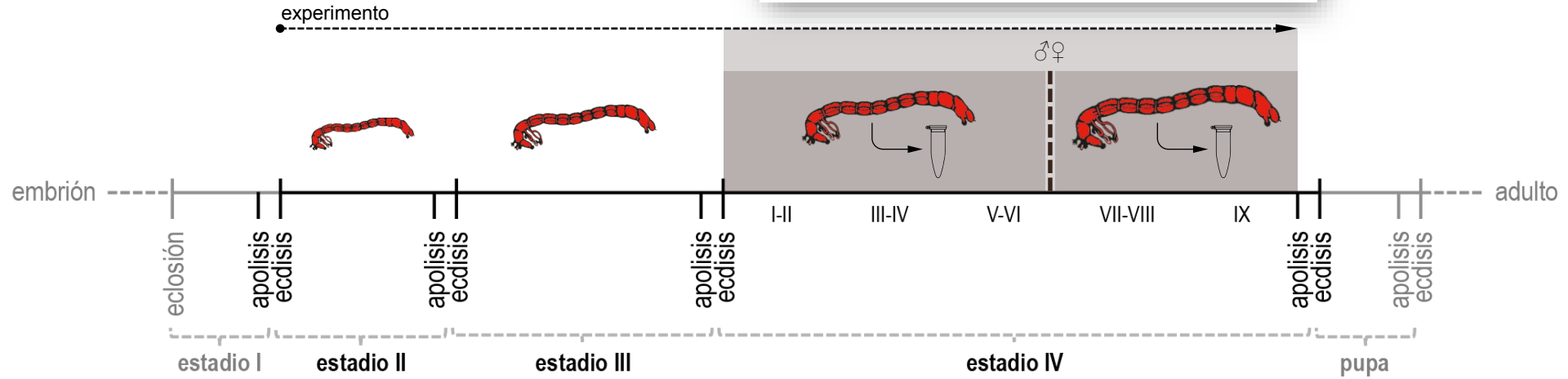
1. This Test Guideline is designed to assess the effects of prolonged exposure of chemicals to the sediment-dwelling larvae of the freshwater dipteran *Chironomus* sp. It is mainly based on the BBA guideline using a sediment-water test system with artificial soil, and water column exposure scenario (1). It also takes into account existing toxicity test protocols for *Chironomus riparius* and *Chironomus tentans* which have been developed in Europe and North America (2)(3)(4)(5)(6)(7)(8) and ring-tested (1)(6)(9). Other well documented chironomid species may also be used, for example *Chironomus yoshimatsui* (10)(11).

OECD/OCDE 233
Adopted: 22 July 2010

THE TESTING OF CHEMICALS

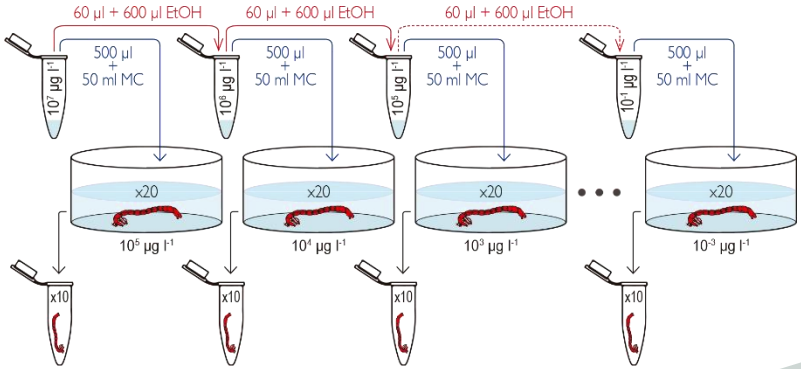
onomid Life-Cycle Toxicity Test
Water or Spiked Sediment

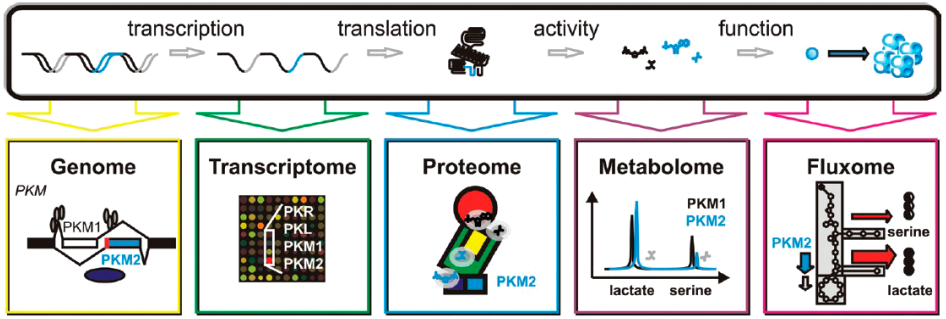
sess the effects of life-long exposure of chemicals on the ering the 1st generation (P generation) and the early part of the 2nd generation (F1 generation). It is an extension of the existing OECD test guideline 219 or 218 using a spiked-water exposure scenario (1) or a spiked sediment scenario (15), respectively. It takes into account existing toxicity test protocols for *Chironomus riparius* and *Chironomus dilutus* (previously named *C. tentans* (2)) that have been developed in Europe and North America (3) (4) (5) (6) (7) (8) (9) and subsequently ring-tested (1) (7) (10) (11) (12). Other well documented chironomid species may also be used, e.g. *Chironomus yoshimatsui* (13) (14). The complete exposure duration is ca. 44 days for *C. riparius* and *C. yoshimatsui*, and –ca. 100 days for *C. dilutus*.



Chironomus riparius

Ftalatos	...
Bisfenoles	Nanomateriales
Filtros solares	...
Metales pesados	Contaminantes emergentes





estudios citológicos

supervivencia

estrés
hsc70
hsp70
...

metabolismo
GAPDH

ribosómico
L4
L13

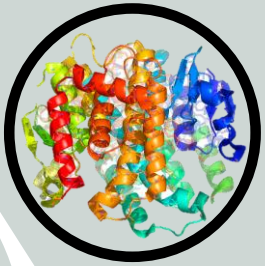
expresión génica

hormonal
EcR
USP
Vg
E75B
Fzt-F1
Dronc

otros
Hb
Fe-L

destoxicación
CYP4G
GST
GPx

actividad enzimática

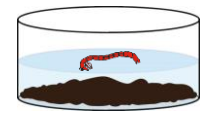


GST
SOD

Chironomus riparius

liofilización

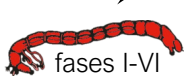
inoculación de larvas de estadio II

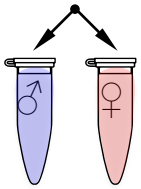


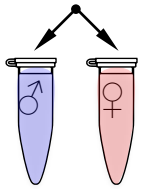
estadio III

estadio IV

recolección

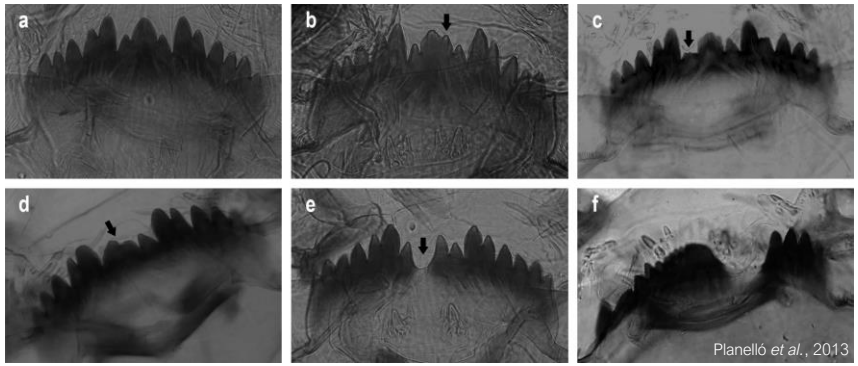




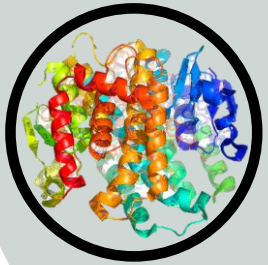


decapitación

deformidades mandibulares

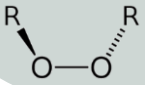


actividad enzimática



SOD

GST



hormonal

EcR

vg

expresión génica

estrés

hsc70

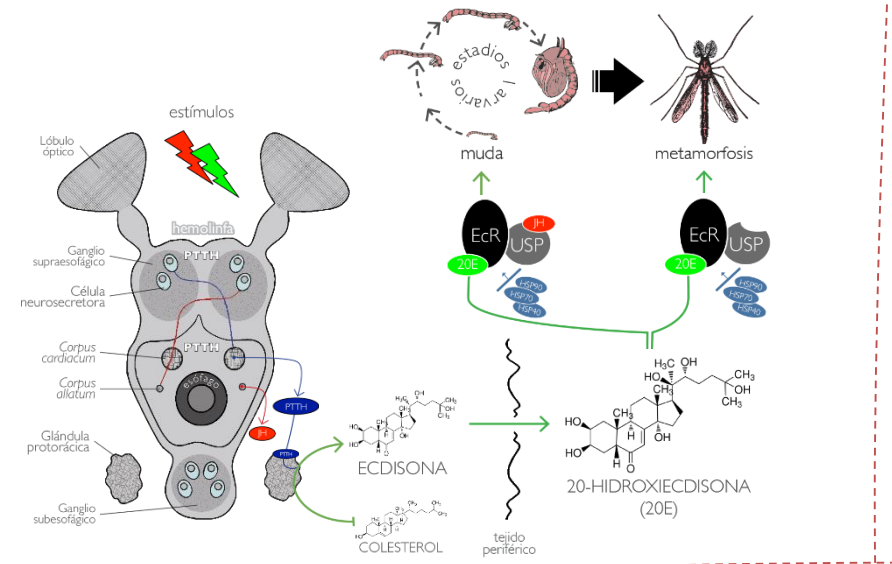
hsp70

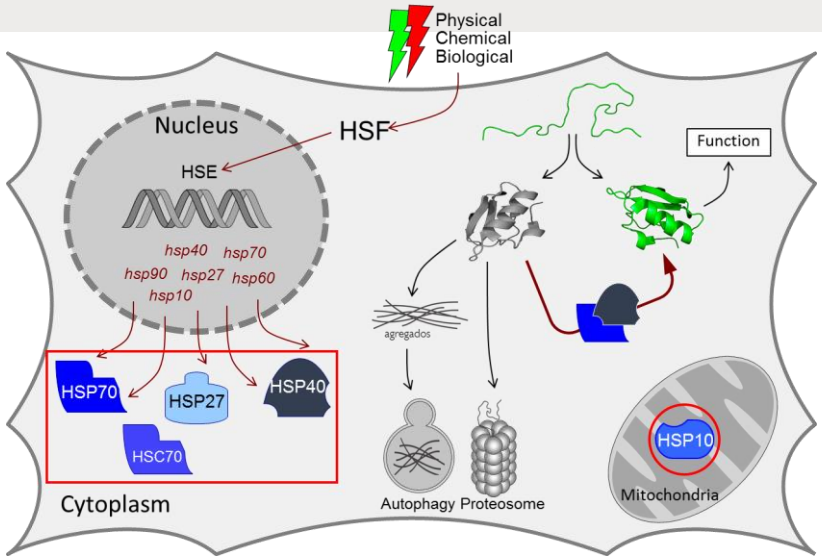
destoxicación

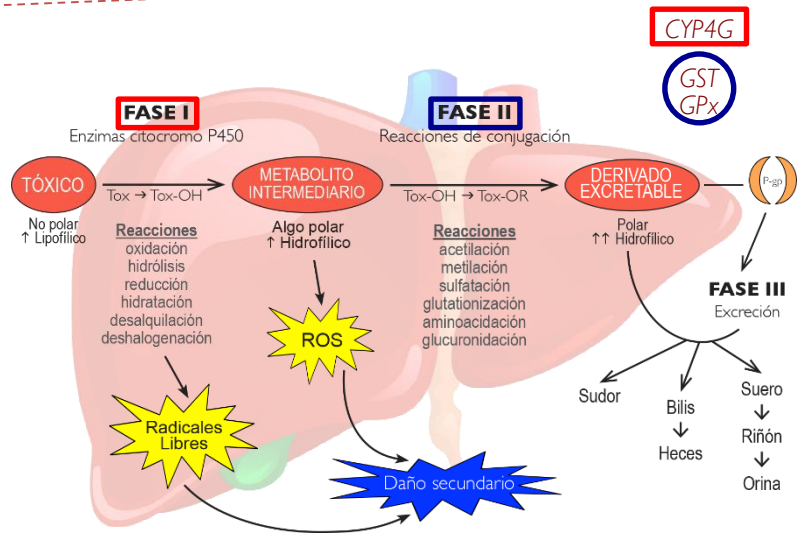
CYP4G

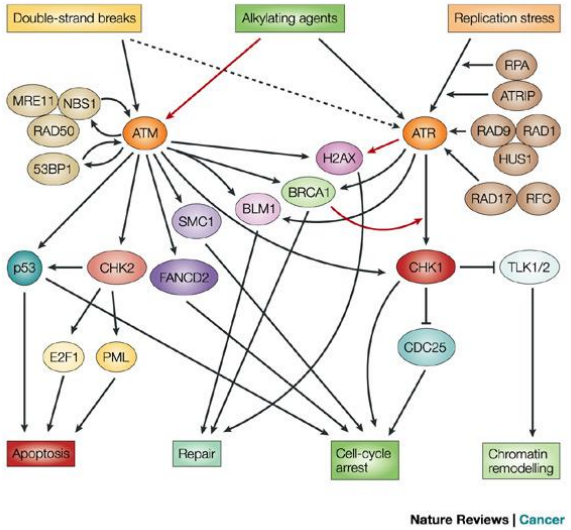
GPx

Relevant pathways









Chemosphere 128 (2015) 266–277

Contents lists available at ScienceDirect

Chemosphere

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

The plasticizer benzyl butyl phthalate (BBP) alters the ecdysone hormone pathway, the cellular response to stress, the energy metabolism, and several detoxication mechanisms in *Chironomus riparius* larvae

Óscar Herrero *, Rosario Planelló, Gloria Morcillo

Grupo de Biología y Toxicología Ambiental, Facultad de Ciencias, Universidad Nacional de Educación a Distancia, UNED, Paseo de la Senda del Rey 9, 28040 Madrid, Spain

PLOS ONE

RESEARCH ARTICLE

Ecdysone-Related Biomarkers of Toxicity in the Model Organism *Chironomus riparius*: Stage and Sex-Dependent Variations in Gene Expression Profiles

Rosario Planelló[1]*, Óscar Herrero[1]*, Pablo Gómez-Sande[2,3], Irene Ozáez[1], Fernando Cobo[2,3], María J. Servia[4]

Aquatic Toxicology 105 (2011) 62–70

Contents lists available at ScienceDirect

Aquatic Toxicology

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

Comparative effects of butyl benzyl phthalate (BBP) and di(2-ethylhexyl) phthalate (DEHP) on the aquatic larvae of *Chironomus riparius* based on gene expression assays related to the endocrine system, the stress response and ribosomes

Rosario Planelló[1], Oscar Herrero[1], José Luis Martínez-Guitarte, Gloria Morcillo*

Grupo de Biología y Toxicología Ambiental, Facultad de Ciencias, Universidad Nacional de Educación a Distancia, UNED, Senda del Rey 9, 28040 Madrid, Spain

PLOS ONE

RESEARCH ARTICLE

Transcriptional deregulation of genetic biomarkers in *Chironomus riparius* larvae exposed to ecologically relevant concentrations of di(2-ethylhexyl) phthalate (DEHP)

Óscar Herrero*, Gloria Morcillo, Rosario Planelló

Grupo de Biología y Toxicología Ambiental, Facultad de Ciencias, Universidad Nacional de Educación a Distancia, UNED, Paseo de la Senda del Rey 9, Madrid, Spain

Chemosphere 144 (2016) 1874–1884

Contents lists available at ScienceDirect

Chemosphere

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

The ribosome biogenesis pathway as an early target of benzyl butyl phthalate (BBP) toxicity in *Chironomus riparius* larvae

Óscar Herrero[1,*], Rosario Planelló[1], Gloria Morcillo

Grupo de Biología y Toxicología Ambiental, Facultad de Ciencias, Universidad Nacional de Educación a Distancia, UNED, Paseo de la Senda del Rey 9, 28040 Madrid, Spain

PLOS ONE

Transcriptional Responses, Metabolic Activity and Mouthpart Deformities in Natural Populations of *Chironomus riparius* Larvae Exposed to Environmental Pollutants

Rosario Planelló,[1]* María J. Servia,[2]* Pablo Gómez-Sande,[3,4] Óscar Herrero,[1] Fernando Cobo,[3,4] Gloria Morcillo[1]

RESEARCH ARTICLE

The BPA-substitute bisphenol S alters the transcription of genes related to endocrine, stress response and biotransformation pathways in the aquatic midge *Chironomus riparius* (Diptera, Chironomidae)

Óscar Herrero[1]*, Mónica Aquilino[1], Paloma Sánchez-Argüello[2], Rosario Planelló[1]

PROYECTOS / TRABAJOS

- 2016-2018. Ecotoxicología y cambio climático: estandarización de bioensayos toxicogenómicos en invertebrados (CTM2015-64913-R).
- 2013-2015. Ecotoxicología de disruptores endocrinos en invertebrados acuáticos: efectos genéticos y epigenéticos (CTM2012-37547).
- 2010-2012. Evaluación del impacto de disruptores endocrinos ambientales en invertebrados bénticos. Identificación de biomarcadores moleculares por medio de tecnologías genómicas en Chironomus riparius como especie modelo en toxicología acuática (CTM2009-07189).
- 2007-2009. Caracterización de biomarcadores de ecotoxicidad acuática en el organismo de referencia Chironomus riparius frente a metales y compuestos orgánicos disruptores endocrinos (CTM2006-12479/TECNO).

PROYECTOS / TRABAJOS

- Estrategia inteligente para la determinación de patrones de respuesta comunes por exposición a contaminantes alteradores endocrinos mediante ensayos multinivel en organismos acuáticos de relevancia ecológica.

PROYECTOS / TRABAJOS

EFFECTS OF FIELD-COLLECTED SEDIMENTS EXPOSURE ON THE HEALTH STATUS OF *CHIRONOMUS RIPARIUS*

Hélène Arambourou[1], Óscar Herrero[2], Lola Llorente[2], Íñigo Moreno[3], Nicolas Delorme[1], Leire Méndez-Fernández[3], Inmaculada Fuertes[4], Carlos Barata[4], Rosario Planelló[2*]

[1] Équipe Écotoxicologie, Irstea – Unité de Recherche Riverly, Villeurbanne Cedex, France.
[2] Biology and Toxicology Group, Science Faculty, Universidad Nacional de Educación a Distancia, UNED, Madrid, Spain. (rplanello@ccia.uned.es)
[3] Dpto. Zoología y Biología Celular Animal, Facultad de Ciencia y Tecnología, Universidad del País Vasco (UPV/EHU), Leioa, Spain.
[4] Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA), Spanish Research Council (CSIC), Barcelona, Spain.

INTRODUCTION

Multidisciplinary approaches provide new insights into the poorly understood relationship between toxic exposure and health status. Our aim was to assess the **effects of heavy metals** contamination on ***Chironomus riparius***, through a multidisciplinary strategy that combines classical ecotoxicological parameters (**life history traits**) with morphological (**shape variations**), physiological (**respiration rate**) and molecular responses (**gene expression, lipidomic**).

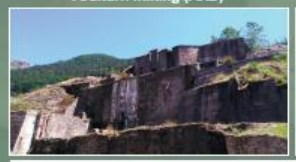
MATERIALS & METHODS

Sediments were collected in the Basque Country (Spain), in an area surrounded by old mines, and were characterized by a **gradient of heavy metal contamination**. Laboratory larvae were used according to toxicity testing guidelines (US-EPA, 1996; OECD, 2001). Individuals were exposed throughout the entire larval cycle (1st – 4th instar) to sediments collected in the field. Markers were measured in larvae and adults. Whole 4th instar larvae were used to analyze by qPCR the transcriptional variations in genes related to **endocrine pathways** (*EcR, InR*), **cell stress response** (*hsp10, hsp70*) and **oxygen transport** (*HbA, HbB*). Head capsules were mounted on microscope slides using using Eukitt® medium.

RESULTS

Midges exposed to the most contaminated sediment showed **delayed emergence** of about 5 days for females and 4 days for males. **Larval mass** and larval **respiration rate** were also **significantly reduced** in that group. Furthermore, the **lipid profile was completely different** in larvae from this sediment with individuals exhibiting more storage lipids. Changes in **transcriptional profile** and **length variations** of larval menta were detected.

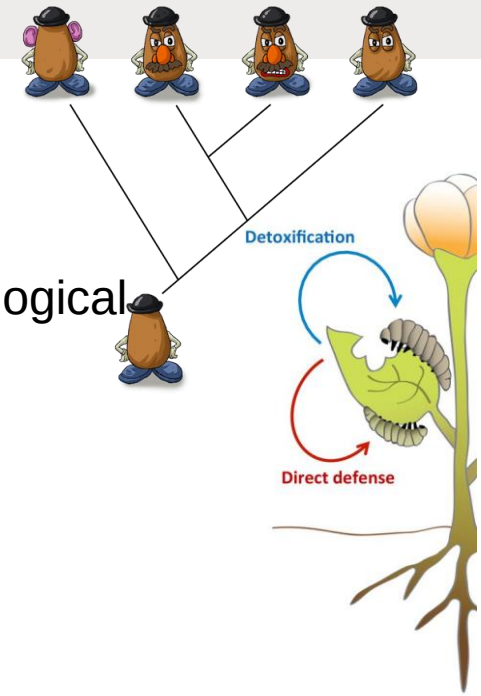
The *EcR* and *InR* genes seems to



Concentrations of Cd, Pb and Zn in the three study sediments

Area	Abbreviation	Cd (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})

I DO NOT WORK IN ECOTOXICOLOGY



¡DO NOT WORRY!

Biomarkers can help you to deep into the endocrine physiological responses of insects **in many diverse situations**.

I WORK WITH A NON-MODEL INSECT

¡DO NOT WORRY!

Biomarkers work also in **non-model insects;** you need more time.

PROYECTOS / TRABAJOS

- Caracterización de nuevos biomarcadores de efecto de contaminantes en el insecto no modelo *Prodiamesa olivacea* (Diptera)

PROYECTOS / TRABAJOS

PRODIAMESA OLIVACEA, A NOVEL NON-MODEL ORGANISM FOR ECOTOXICITY STUDIES IN NATURAL SCENARIOS

Lola Llorente[1], Óscar Herrero[1], Rosario Planelló [1]

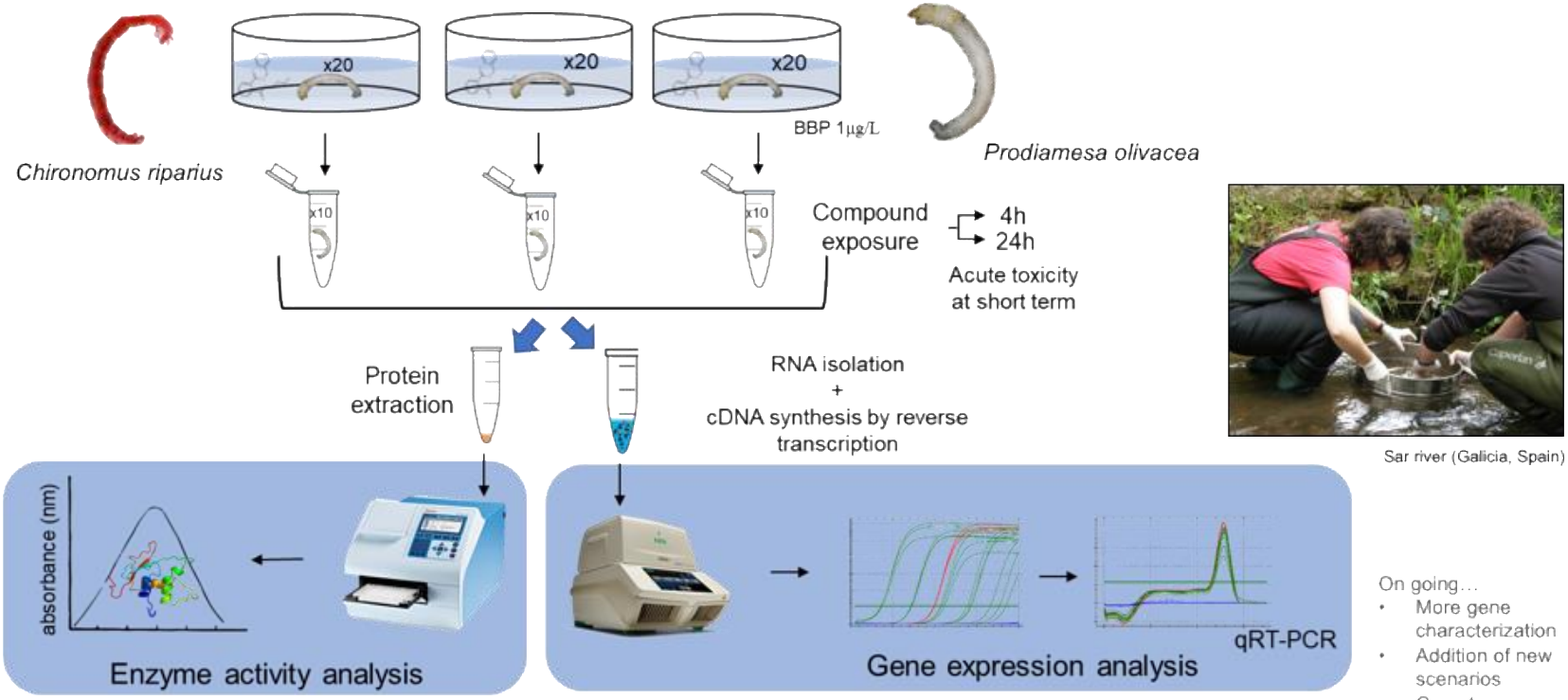
[1]Environmental Toxicology and Biology. Faculty of Sciences. UNED, 28040, Madrid, Spain

4th International Congress on Occupational & Environmental Toxicology

UNED

24th October 2018

Prodiamesa olivacea



Sar river (Galicia, Spain)

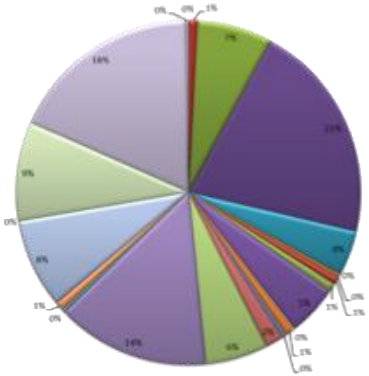
On going…

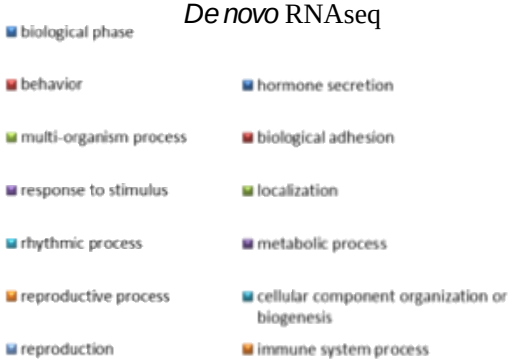
- More gene characterization
- Addition of new scenarios
- Comet assay

Lola Llorente Ortega

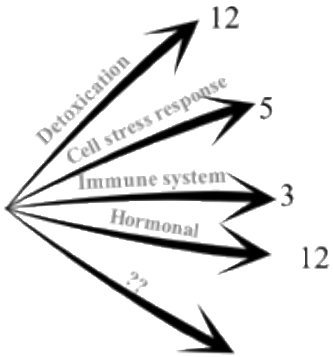
Prodiamesa olivacea

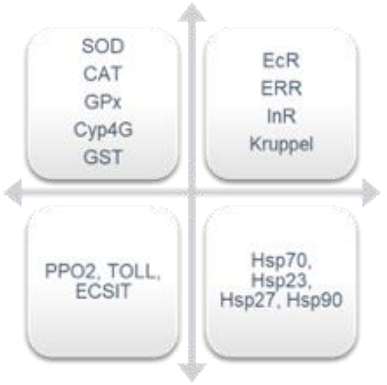
Prodiamesa olivacea





De novo RNAseq





De novo gene identification and characterization

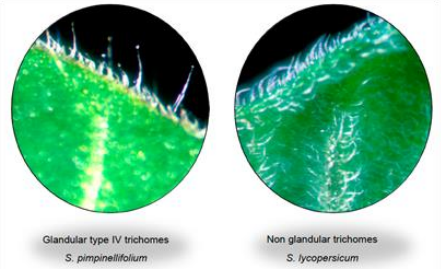
1. Searching of genes used as biomarkers on the transcriptome
2. Conserved regions
3. Multiple alignment
4. Phylogenetic tree

PROYECTOS / TRABAJOS

- 2016-2018. Defensas mediadas por tricomas glandulares en tomate: factores de inducción, caracterización e implicaciones en el control biológico de pulgones (Aphididae) (AGL2015-67733-R).

- 2018-2020. Evaluación del potencial biocida de las metilcetonas y posibles implicaciones de su uso en el manejo integrado de plagas (UNED Independent Thinking).

Exploit tomato natural defences



PROYECTOS / TRABAJOS

Nematology
15:45

ANALYSIS OF PARTIAL RESISTANCE TO FOLIAR HERBIVORES AND *MELOIDOGYNE INCOGNITA* IN WILD AND MEDITERRANEAN TOMATO ACCESSIONS

E. DE LA PEÑA[1,2], L. BLANCO[2], J. ANTONIO DÍAZ-PENDÓN[2], Ó. HERRERO[3], R. PLANELLÓ[3], R. FERNÁNDEZ-MUÑOZ & V. FERRERO[2,4]

[1] Ghent University, Department of Biology
K.L. Ledeganckstraat 35, BE-9000 Ghent, Belgium
[2] Instituto de Hortofruticultura Subtropical y Mediterránea, IHSM-UMA-CSIC
Finca La Mayora, 29750 Algarrobo-Costa, Spain
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A commonly invoked hypothesis explaining the high susceptibility of many crops to pests and diseases is that crops in the process of domestication have lost defensive traits that result in a decreased resistance to pests and diseases. At the same time, ecological theory, based on the molecular cross-talk regulating defensive responses of plants towards insect pests and diseases, predicts the occurrence of resistance trade-offs i.e. the resistance to a certain pest goes at the cost of the resistance to a different pest. To test these two hypotheses, we conducted a combination of field and greenhouse experiments using 24 genotypes of tomato and a selection economic relevant pest in subtropical and Mediterranean areas. We used wild tomato species and traditional Mediterranean cultivars from different geographic origins; all of them representing different stages in the primary and secondary domestication of tomato. Moreover, we included in the study accessions carrying the Mi-1 resistance gene to address not only the potential resistance of these accessions to root-knot nematodes but also to foliar insect pests. We compared the performance of three different pests, the aphid *Macrosiphum euphorbiae,* the cotton leafworm, *Spodoptera littoralis*, and the root-knot nematode *Meloidogyne incognita.* The results of the experiments show strong differences in the performance of pests according to tomato provenance. The detected partial resistances of tomato accessions to foliar pests and nematodes do not seem to be correlated with the degree of domestication. The implications of these findings to understand plant-pests interactions during the domestication process are further discussed.

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2-DODECANONE: A POTENTIAL ALTERNATIVE TO SYNTHETIC INSECTICIDES?

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Glandular trichomes are specialized epidermal protuberances found on the surface of about 30% of all vascular plants, that produce and secrete structurally diverse specialized metabolites. Among the many substances synthesized by trichomes for plant defense purposes, methyl ketones are a widely-produced group of chemicals and their insecticidal efficacy has been described against some arthropods, such as aphids or spider mites. However, information about the mode of action and molecular effects of these compounds on insects are still very scarce. In the present study, the toxicity of 2-dodecanone (CAS 6175-49-1) was investigated in *Chironomus riparius* aquatic larvae, an insect model species commonly used in ecotoxicity studies. The effects of acute 24-h and 96-h exposures to 0.05 µg/L, 5 µg/L and 500 µg/L 2-dodecanone, were evaluated at the molecular level by analysing changes in the transcriptional rate of genes related to the endocrine system (EcR, ERR, Vtg and Cyp18a1), detoxication pathways (Cyp4G, GST and GPx) and the energy metabolism (GAPDH). Ribosomal gene 26S, actin and ribosomal protein L13 were used as reference genes. Our results showed that 2-dodecanone caused a clear dose- and time-dependent toxicity in most of the selected genetic biomarkers. While significant effects were detected even after 24-h acute exposures, longer treatments (96h) triggered a general transcriptional repression in most of the analysed genes. This study provides novel and interesting results in *C. riparius* on the toxic effects of an isolated secondary metabolite, naturally present in plants, and highlights the potential suitability of this organism to delve into the molecular effects of plant defences in insects. These findings provide new insights into insecticidal efficacy of 2-dodecanone, which might be explored under field conditions for plant protection and pest management, to reduce reliance on synthetic pesticides.

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Key words: genetic biomarkers; *Chironomus riparius*; toxic effects; plant defense; pest management

2-DODECANONE: A POTENTIAL ALTERNATIVE TO SYNTHETIC INSECTICIDES?

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INTRODUCTION

Glandular trichomes are specialized epidermal protuberances found on the surface of about 30% of all vascular plants, that produce and secrete structurally diverse specialized metabolites. Some of these compounds are toxic substances that compromise insect survival, and may delay their growth and pupation. Among the many substances synthesized by trichomes for plant defense purposes, **methyl ketones** are a widely produced group of chemicals and their insecticidal efficacy has been described against some arthropods, such as aphids or spider mites. However, information about the mode of action and molecular effects of these compounds on insects are still very scarce.

In the present study, the toxicity of **2-dodecanone** (CAS 6175-49-1) was investigated in ***Chironomus riparius*** aquatic larvae, an insect model species commonly used in ecotoxicity studies. The effects of acute 24-h and 96-h exposures to 0.05, 5, and 500 µg/L 2-dodecanone, were evaluated at the molecular level, throught **genetic biomarkers**.

Embryo, larvae and pupae, the aquatic stages of *Chironomus riparius* life-cycle.

MATERIALS & METHODS

Larvae were reared under standard laboratory conditions, according to toxicity testing guidelines (US-EPA, 1996; OECD, 2001). Whole larvae were used for RNA extraction and quantitative real-time PCR was used to evaluate the mRNA expression profile of genes selected during fourth instar larvae.

Changes in the transcriptional rate of different genes were investigated, related to: 1) the endocrine system (***EcR***, ***ERR***, ***Vtg*** and ***Cyp18a1***); 2) detoxication pathways (***Cyp4G***, ***GST*** and ***GPx***) and 3) the energy metabolism (***GAPDH***). Ribosomal gene *26S*, *actin* and ribosomal protein *L13* were used as reference genes.

PROYECTOS / TRABAJOS

- La zona híbrida *de Chorthippus parallelus*: una ventana a las bases genéticas de la formación de especies.

PROYECTOS / TRABAJOS

Evaluation of the physiological response of *Chorthippus parallelus* to *Wolbachia* infection: characterization of new molecular biomarkers

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INTRODUCTION

Chorthippus parallelus parallelus (Cpp) and ***Chorthippus parallelus erythropus*** (Cpe) are two grasshopper subspecies whose distributions overlap in the Pyrenees. They form a **hybrid zone** at those points where the topography and their ecological requirements allow. The morphological, physiological, genetic and behavioural differences between these subspecies (and their natural and laboratory-reared hybrids) have been intensively studied in recent years, for which reason this hybrid zone is considered a singular **model in Evolutionary Biology**. They offer an excellent panorama of evolution 'in action'.

On the other hand, ***Wolbachia*** is a genus of obligate endosymbiont bacteria that induces changes in the reproduction of arthropods and nematodes. In previous studies we have reported the existence of these bacteria in individuals and populations of *C. parallelus*. A wide-ranging survey of *Wolbachia*-infected European populations of *C. parallelus* has been carried out in recent years showing a remarkable biogeographical distribution of subspecies-specific bacterial strains, this including recombinant *Wolbachia* appearing just in the hybrids. Even more interesting is the evidence of **cytoplasmic incompatibility** affecting this model (i.e. a very significant reduction of descendants in certain crosses between infected and uninfected grasshoppers), that could be acting as a **reproductive barrier** affecting the hybrid zone. *Wolbachia* effects in the male gametogenesis of these organisms have also been evidenced as significant variation in the production of abnormal spermatids and in the chiasmata number during meiosis. Studies in other organisms confirm that this talented bacterium affects trough uncertain physiological ways not only the reproduction and fecundity of the infected individuals, but also their behaviour.

MATERIALS & METHODS

Samples were collected from natural populations of grasshoopers in the **Pyrenees** (Spain). Individuals were dissected and **different tissues** were kept for RNA extraction. Adults, **males and females, infected and uninfected** by *Wolbachia* were selected for this study, and our preliminary work focused on gonadal tissue.

Chorthippus parallelus (female on the left) and the endosymbiont *Wolbachia* (center, right) were studied in this work.

Genes -commonly used as **molecular biomarkers** in other insects species- were *de novo* identified in *C. parallelus*: 1) ***EcR***, *coding for* ecdysone receptor; 2) ***hsp70*** and ***hsc70***, involved in cell response to stress and also chaperons involved in the EcR folding and 3) ***GPx*** (glutathione peroxidase), involved in biotransformation pathway. Quantitative **real-time PCR** was used to evaluate the mRNA expression profile of genes selected. Ribosomal gene *26S*, *GAPDH* and *actin* were used as reference genes.

Molecular biomarkers are useful as an aid to **identifying the causes** of observed population- and community-level effects.

Multidisciplinary approaches -including **molecular biomarkers-** may be **useful in identified endocrine disruptors** to protect invertebrate populations and as screening systems for vertebrates.

From the ongoing interplay of these modern tools of science, **new insights** into initial **mechanisms of chemical-induced toxicity** have been gained.

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Biomarcadores moleculares para la evaluación de la respuesta tóxica en invertebrados modelo y no modelo

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