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THE ROLE OF SCIENCE IN ENVIRONMENTAL DECISION-MAKING



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# Abstract Book

## Buenos Aires 2015

**SOCIETY OF ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY**  
Environmental Quality Through Science®

*M.J. Damonte, M.B. Poliserpi, INTA; M.F. D'Andrea, J.C. Brodeur, CONICET INTA*

Se evaluó en campos de soja los efectos de la aplicación del insecticida ENGEO® (Lam: Lambdaialotrina 10,6% p/v + Tia: Tiametoxam 14,1% p/v) sobre *Leptodactylus latrans*, *Leptodactylus latinasus* e *Hypsiboas pulchellus*. El muestreo se realizó 15 días antes y 2 y 4/5 días después de la aplicación del insecticida en tres lotes de soja de Bs. As. (L1, L2 y L3). Además se muestreó la reserva "El Destino" como sitio control. En todos los sitios y en las diferentes fechas se capturaron ranas y se tomaron muestras de suelo y agua. Se evaluó la condición corporal y biomarcadores de exposición a xenobióticos (ChE: colinesterasas y GST: glutatión-S-transferasa) o de estrés oxidativo (CAT: catalasa y GSH: glutatión reducido). Las muestras de suelo y agua no contenían Tia ni Lam antes de la aplicación. Después de la aplicación, se detectó Engeo en el suelo de L2 (Tia 190 ppb, lam 231 ppb) pero no en los otros dos lotes, probablemente debido a la gran cobertura vegetal de estos sitios. La condición corporal de *L. latinasus* estuvo reducida con respecto al control en todos los lotes siendo significativa en L3. No hubo diferencias entre los sitios cultivados y control para *L. latrans*. *H. pulchellus* presentó un significativo aumento de la condición corporal. La actividad de ChE fue significativamente reducida dos días después de la aplicación para *L. latinasus* en L1 y L2 e *H. pulchellus* solo L2, recuperando sus niveles basales 4 días después. Lo mismo ocurre con CAT, dos días después de la aplicación, sus valores se reducen significativamente para *L. latinasus* en L1 y L2. Los niveles de actividad de GST fueron significativamente menores para *L. latrans* en L1 y L3 con respecto al control, tanto antes como después de la aplicación. Este trabajo es el segundo en reportar una inhibición de la GST en *L. latrans* de zonas agrícolas de la región pampeana. La mayor cantidad de alteraciones encontradas en *L. latinasus* podrían deberse a que es una especie exclusivamente terrestre, estando más expuesta a la aplicación de plaguicidas. En este estudio, se demuestra la importancia de muestrear inmediatamente después de la aplicación de este formulado.

**PT61. Analysis of the take-back program for empty pesticide containers in the municipality of Bom Repouso, mg, brasil**

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Brazil is the first in the ranking of take - back program for empty pesticide containers comparing the returns with the amount of pesticides placed on market since 2011, reaching a percentage of 96% of return in 2013. The Brazilian take-back program was originally designed targeting large rural areas, which may not apply to small rural communities, generally more isolated and with poor infrastructure, that must require large storage and transport costs. Therefore, it is still very common to burn and bury empty pesticides containers in this areas, such as storing it in inappropriate places or leaving it in the field. Thus, this research aimed to analyze the efficiency of empty pesticide containers reverse logistics process in small Brazilian agricultural counties, taking as a case study the municipality of Bom Repouso, MG, Brazil, which is characterized by the production of strawberry and potato on properties with family labor and intensive use of pesticides. Therefore, it was made a comparative analysis of the amount of packaging sold in the city and the amount returned to the place where occurs the management of the empty containers that it's under INPEV's responsibility, called Central de Pousos Alegres, in MG, Brazil, between the years 2012 and 2014. We compared the total amount of returned packaging with the sales for the seven best-selling pesticides in the city. Considering the requirement of the law that farmers must return empty pesticides containers in a maximum of one year after purchase, it was taken a comparison between two periods, from the year 2012 to 2013 and 2013 to 2014. In both periods analyzed, the amount of packaging sold, considering only the seven most traded products, it was much higher than the returned packaging, reaching a difference of up to 67% between the product with the highest sales volume and the total amount of returned packages. Besides, there was a decrease of the total quantity of empty pesticides containers returned from 2013 to 2014, from 156,29 kg to 62,76 kg. Hard storage for empty pesticide containers and transportation costs were the main points highlighted by dealers as reasons given by producers for not returning and, consequently, improperly dispose such waste. It is believed that simple solution as the itinerant collection, can help to improve this scenario and ensure proper disposal of empty pesticides containers. Key-words: pesticides, small rural communities, waste handling, waste recovery.

**PT62. Efficiency of Biobeds for pesticide disposal: Ecotoxicity evaluation using *Collembola* reproduction tests**

*L. Camargo, Soil Department; O. Filho; L.C. Filho, UDESC / Soil Department; L. Gebler, Embrapa; J.C. Niemeyer, Universidade Federal de Santa Catarina*

Pesticide waste disposal from washing the equipment consist in a source of contamination for agricultural soils. An alternative to this problem was founded in some countries: the discard is done in reactors filled with straw, peat and soil mixed, called Biobeds. In Brazil, this sort of effluent is not remarkable. In South of Brazil, Mancozeb and Chlorpyrifos are used in apple cultures, but their waste is discharge in soil and effects on soil organisms are unknown. An experiment with Biobeds was installed on the Experimental Station of Embrapa, at Vacaria City, in the apple harvest between 2013-2014, aiming to determine the toxicity into a biobed and in a local natural soil – Oxisol, during the harvest. The reactors were water boxes with biomix (Biobed) or Oxisol. The boxes received 50 L of effluent for each application in the crop. Samples of biomix and soils were collected on time 0 (without pesticide), 90, 150, 210 and 240 days after the first pesticide application, and ecotoxicity reproduction tests were conducted with *Collembola* (*Folsomia candida*) following ISO 11267 (2014). The number of juveniles for each treatment (Biomix or Oxisol) was compared for every time, with t-test. The collembolas not survive in any treatment for two first samplings. Only the last one(420 days) there was reproduction, and this not diverged statically from control, suggesting a toxicity reduction throughout time. Whereas, when compared Biomix against Oxisol, reproduction rates for the first one was upper than second. Although absence of toxicity in natural soil reactors after a period, in a natural system, discharge a substance which have an unknown toxicity and behavior, could impact soil organisms promoting a dissipation from that area, increasing the contamination already high, due lack of the pesticide dissipation. The study points to the need to know the toxicity of this waste, so common and generated in high amounts by agricultural activities and still without specific legislation in Brazil.

**PT63. Assessing risk in the age of global trade: a comparison of regulatory bee requirements on plant protection products in South America, USA and Europe**

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Are the regulations governing the registration of plant protection products (PPPs) in developing countries just copied from their counterparts in the industrialized nations? Are the current and emerging agricultural superpowers in South America (e.g. Brazil) adapting their regulatory standard to the agricultural markets of the USA and the European Union? What about issues relevant to environmental, consumer and farmer protection in the countries of origin: are standards the same as in the USA or the EU? We take a closer look at the registration process of different regions across the globe (South America, U.S. and Europe). Taking the risk assessment of bees as the basis for a comparison, we identify the specific regional data requirements and risk assessment approaches for registration of PPPs. We then discuss the implications of South America's eco-climatic variability and agricultural diversity with respect to the assessment of potential risk associated with PPPs on bees. Finally, we identify global trends in regulatory requirements and discuss the advantages and possible drawbacks of setting common approaches to evaluate risk and for streamlining the registration process.

**PT64. Biomarkers of oxidative damage and its relationship with risk factors in a locality environmentally exposed to agrochemicals**

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In Santa Fe province, located in the center of Argentina, has been historically one of the main agricultural centers. The production of the main crops (soybean, sunflower, corn, wheat and sorghum) significantly increased in recent years. The aim of this study was to evaluate oxidative damage generated by environmental exposure to agrochemical mixtures in people living in Santo Domingo, Santa Fe, Argentina and to compare them with healthy non-exposed people from Santa Fe city. The study involved 72 subjects, 44 from Santo Domingo (rural area) and 28 healthy donors from Santa Fe city (urban area). Samples were taken at two different moments, in September (low pesticide spraying) and in March (post-period of high spray). The activity of Catalase (CAT), Superoxide dismutase

The contamination of bovine milk with pesticide residue is an extremely troubling issue, as milk is one of the most consumed foods of animal origin in the world. It is widely consumed during childhood, as it is essential for a child's survival. Thus, the quality control of milk is of critical importance. The pyrethroid pesticides in Brazil are widely applied to livestock to combat tick (*Boophilus microplus*) and horn fly (*Haematobia irritans*) ectoparasites. Although the application of most pyrethroids in cattle is done using the pour-on method, they may cross the skin barrier, enter the blood stream and can be excreted into milk due to lipophilicity of pyrethroids associated with the high fat content of milk. The pyrethroids are liposoluble molecules; thus, they tend to migrate to the lipid portion of the food. Therefore, it is suspected that pyrethroid residues can contaminate bovine milk, of which, detection and quantification are not easy tasks, especially at low concentrations in both complex and fatty matrices. Extraction of pesticides from fatty foods typically requires several cleanup steps in order to minimize the amount of co-extractives, and to isolate the targeted pesticides for analysis to yield more reproducible results and preserve the instrument's integrity. Our analytical method is based on the following steps: solvent extraction using lyophilized samples and ultrasound; cleanup with a cartridge of basic alumina in tandem with a C18 cartridge; and analysis using gas chromatography (GC) and tandem mass spectrometry (MS/MS) in negative chemical ionization (NCI), which can be used to detect 17 pyrethroids (transfluthrin, allethrin, prallethrin, imiprothrin, resmethrin, teramethrin, bifenthrin, phenothrin, cyhalothrin, permethrin, cyfluthrin, cypermethrin, fenvalerate,  $\tau$ -flualinate, deltamethrin, kadethrin and flumethrin) and one organophosphorus pesticide (chlorpyrifos). Surrogate standards d10-Chlorpyrifos, d6-trans-permethrin and d6-trans-cypermethrin were employed for quantitation by isotopic dilution method. The objectives of this study were to quantify 17 pyrethroid and chlorpyrifos residues in 20 samples of bovine milk from southern Brazil, and to evaluate the presence of cypermethrin in bovine milk in cattle exposed to cypermethrin from the pour-on method. Pyrethroids were detected in all the samples at concentrations ranging from 0.47 to 270 ng g<sup>-1</sup> lipid weight (LW). Cypermethrin was the predominant pyrethroid.

#### WP111. Ecotoxicity evaluation of Biobeds for pesticide disposal using Enchytraeids reproduction tests

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Pesticide waste disposal from washing the equipments consist in a source of contamination for agricultural soils. An alternative to this problem was founded in some countries: the discard is done in 'beds' filled with straw, peat and soil mixed, called Biobeds. In Brazil, this sort of effluent is not remarkable. In South of Brazil, Mancozeb and Chlorpyrifos are used in apple cultures, but their waste is discharge in soil and effects on soil organisms are unknown. An experiment with Biobeds was installed on the Experimental Station of Embrapa Vacaria in the apple harvest between 2013-2014, aiming to determine the toxicity of biobed and a natural soil – Oxisol during the harvest. The biobeds were water boxes with biomix or oxisol. The boxes received 50 Liters of effluent for each application in the crop. Samples of biomix and soils were collected on time 0 (without pesticide), 90, 150, 210 and 240 days after the first pesticide application, and ecotoxicity reproduction tests were conducted with *Enchytraeus crypticus* (Enchytraeids) following ISO 16387:2014. The number of juveniles for each treatment (Biomix or Oxisol) was compared for every times, with t-test. The last sample in biomix (420 days after the first application) not differed from the control ( $p > 0,05$ ), showing a reduction of toxicity in the course of time. However, in Oxisol, the toxicity remained over time. Furthermore, reproduction of *E. crypticus* was lowest in Oxisol than in biomix over the sampling time, which indicate higher pesticide toxicity for *E. crypticus* in Oxisol. The study indicates that biobeds can be used for reduce the washing and handling pesticide contamination for agricultural areas in Brazil.

#### WP112. Ecotoxicity of Chlorpyrifos to *Folsomia candida* (COLLEMBOLA) and *Ensenia andrei* (OLIGOCHAETA) in two different Brazilian soils.

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Apples orchard have high representativeness in Brazilian south and for this cultivation, several pesticides are used, specially the insecticide Chlorpyrifos. This product is forbidden in some countries, whereas used in many crops in other ones. For pesticide registration in Brazil, earthworms lethality test is the only representative of soil fauna. The aim of this study was measure the toxicity of Chlorpyrifos to collembolan (*Folsomia candida*) and earthworms (*Ensenia*

*andrei*) using standardized ecotoxicity tests following the ISO 11267 (2014), ISO 11268-1 and ISO 11268-2 (2012), respectively, in two representative soils for apple cultivation in the South – Oxisoils (Latossolo and Nitossolo). The results showed high toxicity for collembola in both soils in lethality tests ( $LC_{50} < 1$  mg kg<sup>-1</sup>) and for reproduction tests ( $EC_{50} < 0,005$  mg kg<sup>-1</sup>). The toxicity in latossolo and nitossolo did not differ statistically for the reproduction of earthworms ( $EC_{50}$ : 6,2 mg kg<sup>-1</sup> and  $EC_{50}$ : 5,9 mg kg<sup>-1</sup> respectively), and did not showed high toxicity in lethal tests ( $LC_{50}$ : 64,09 mg kg<sup>-1</sup> and  $LC_{50}$ : 204,63 mg kg<sup>-1</sup> respectively). Although the Brazil's law indicate only lethality test with earthworms to the pesticides registration, this study showed high toxicity of Chlorpyrifos to collembolan – representative of soil mesofauna in studies with pesticides in Europa. The results also evidenced the importance of sublethal tests in the evaluation of pesticides toxicity.

#### WP113. Evaluation of endotoxin *Bacillus thuringiensis* var. *israelensis* and pyrethroid cypermethrin in *Chironomus calligraphus* (Diptera: Chironomidae)

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Pesticides are extensively used for the control of agricultural pests and disease vectors but they also affect both humans and animals. Cypermethrin (CYP) is a pyrethroid widely used in the Pampean region. Bioinsecticides have received great attention as environmentally benign and desirable alternatives. *Bacillus thuringiensis israelensis* (Bti) is one of the most used bioinsecticides for the control of mosquitoes and agricultural activities. In ecotoxicology, *Chironomus* species are widely used as bioindicators of the effects of chemicals in aquatic ecosystems. In order to compare the toxicity of different pesticides, *Chironomus calligraphus* was selected, which is highly sensitive to heavy metals and pesticides. Due to its small size, short generation time and easy maintenance in laboratory conditions, this species could be useful as a potential bioindicator of the Pampean region. Third instar larvae were exposed to serial dilutions of CYP (0.0025-6.4 µg/L) and Bti (0.5-16 µg/L) to determine 96-h  $LC_{50}$  values. A control group without insecticide was included. After the 96 h assays, the entire larvae were fixed in Bouin solution and 4% formaldehyde, then dehydrated and embedded in plastic resin. Sections 3 µm were stained with hematoxyline-eosine and observed under optical microscope. For the ultrastructural studies the larvae were fixed in 2.5% glutaraldehyde and ultrathin section were observed under electron microscope. The 96-h  $LC_{50}$  values were 1.506 µg/L and 0.045 µg/L for Bti and CYP respectively. We focus in the histological and ultrastructural alteration in the mid section of the digestive tract. The midgut of the control group showed a single layer of cubical cells with microvilli in the apical surface and a big central nucleus. At very low concentration of Bti (2 µg/L) no histological changes were observed, but at 4 ng/ml exposure some minor histopathological changes were observed as disruption of the microvilli. At Bti exposures of 7.5 µg/L, the midgut cells lost the contact with their basal membrane and started to be disorganized, while at 15 µg/L the epithelium is completely disorganized. *Chironomus calligraphus* was sensitive to Bti and CYP toxicity as other *Chironomus* species. It would be interesting to carry out studies in larvae collected from freshwater bodies with insecticides to confirm the relationship between the stress situations and the histopathological changes observed in this study.

#### WP114. Experimental factorial design for determination of organochlorine pesticides collected in passive samplers with polyurethane foam

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The organic persistent pollutants can be collected through different types of samplers, among them are the passive samplers. They does not need electrical energy and are based in the free flux of the analytes to the sorbent surface. This is made of polyurethane foam (PUF) where the compounds are deposited. In this study, we focused on organochlorine pesticides (OPs) collected during a long exposure time (90 days). The aim of this study is to show the results from an optimized methodology to determine OPs from PUF. The conventional methodology is by Soxhlet (TO-10A Method, EPA). We extracted PUF spiked