PS 1344 CHARACTERIZATION OF DIFFERENTIAL EFFECTS OF ALLETHRIN ON N- AND L-TYPE NEURONAL VOLTAGE-GATED CALCIUM CHANNELS IN DIFFERENTIATED PC12 CELLS.

A. P. Neal[1], S. M. Fox[1], D. Wiwatratana[2], Y. Yuan[1] and W. D. Atchison[1]. *[1]Pharmacology and Toxicology, Michigan State University, East Lansing, MI and [2]Neuroscience Program, Michigan State University, East Lansing, MI.*

Pyrethroid insecticides are widely used world-wide in agriculture, malaria control, and as topical insect repellents. Several studies have identified effects on neuronal voltage-gated calcium (Ca^{2+}) channels (VGCCs). Whole-cell voltage-clamp electrophysiology in differentiated PC12 cells was used to determine the IC_{50}/EC_{50} of effects by a well-characterized type I pyrethroid, allethrin (AL) on N- and L-type VGCC subtypes (Cav2.2 and Cav1.2, respectively). Pharmacological agents were used to isolate specific currents. AL exhibited a concentration-dependent inhibition of peak and end N-type VGCC current with IC_{50}'s ~ 10 μM. In contrast, it caused U-shaped concentration-dependent effects on L-type VGCCs, resulting in L-type current peak and end stimulation with EC_{50}'s in the mid-pM range. At higher concentrations (>50 μM), AL did not stimulate L-type current. Additionally, AL altered the V_{50} of activation, but not inactivation, of L-type VGCCs in a concentration-dependent manner, and altered the kinetics of activation and inactivation of both channel subtypes. AL also significantly increased the Ca^{2+} influx associated with L-type VGCC activation, determined by measurements using the ratiometric dye Fura-2. Finally, we observed that modulation of N- and L-type VGCC by AL can have effects on VGCC expression; a 24 hr incubation with 1 μM AL resulted in increased N-type and decreased L-type VGCC expression visualized by immunocytochemistry. Together, these data demonstrate that AL can differentially modulate VGCC function, resulting in altered Ca^{2+} influx, and may have long term effects on VGCC expression. [Supported by NIEHS R01-ES03299]

PS 1345 PYRETHROID EFFECTS ON MUSCARINIC ACETYLCHOLINE RECEPTOR EXPRESSION IN N1E-115 AND PC12 CELLS.

J. M. Diaz Otero[1], A. P. Neal[2] and W. D. Atchison[2]. *[1]University of Puerto Rico at Cayey, Cayey, Puerto Rico and [2]Pharmacology and Toxicology, Michigan State University, East Lansing, MI.*

Pyrethroid insecticides (PYRs) are widely used and are classified as type I and type II agents. Both primarily target the voltage-gated Na^+ channel, but some studies identified alternative targets of PYRs. Changes in the expression of muscarinic acetylcholine receptors (mAChRs) are observed during developmental PYR exposure. These studies were contradictory; some observed an increase and others a decrease in mAChR expression. We previously observed that PYRs differentially modulate voltage-gated Ca^{2+} channel subtypes, so hypothesized that these discrepancies may be due in part to differential actions on mAChR subtypes. We examined M1 and M2 mAChR expression using fluorescent immunocytochemistry in differentiated (DIF) and undifferentiated (UNDIF) rat pheochromocytoma (PC12) and mouse neuroblastoma (N1E-115) cells. Both models were exposed to the type I and type II PYRs allethrin (AL) and deltamethrin (DM), respectively. Fluorescent staining indicated the expression of surface and cytosolic mAChRs. Both PC12 and N1E cells exhibited differentiation-dependent and -independent effects. In PC12 cells, AL or DM decreased surface M2 expression in UNDIF cells but increased it in NGF-DIF cells. Only DM affected M1 expression in PC12 cells, regardless of differentiation. Conversely, N1E M2 expression was affected in a differentiation-independent manner; both UNDIF and DIF N1E cells had increased M2 expression after DM exposure. However, the effects of AL on M1 expression in N1E cells were dependent on differentiation. In UNDIF N1E cells, M1 expression was unaffected by PYR exposure. In DIF N1E cells, M1 expression significantly increased after exposure to AL, but not DM. Thus, M1 expression in DIF cells of both lines was altered by opposite PYR classes. This suggests that effects of PYRs on mAChR expression are influenced by developmental stage and that these insecticides can exhibit class-specific effects on mAChR subtypes. [Supported by 2R01ES03299 and 1R25NRS06577]

PS 1346 TIME, DOSE, AND STRUCTURE DEPENDENT ACTIONS OF PYRETHROID INSECTICIDES ON RAT THERMOREGULATION.

A. M. Pato[1], C. Sosa-Holt[1] and M. J. Wolansky[1, 2, 3]. *[1]Biological Chemistry (Toxicology), University of Buenos Aires School of Science, Buenos Aires, Argentina, [2]Argentine NRC, Buenos Aires, Argentina and [3]ARG National Agency for Scientific and Technological Promotion, Buenos Aires, Argentina.*

Pyrethroid insecticides produce changes in thermoregulatory response (TR) in small rodents. In rat, type I-pyrethroids permethrin (PM) and bifenthrin (BIF) produce hyperthermia, and type II cypermethrin (CPM) and deltamethrin (DLM) produce hypothermia. These structure-specific TR effects have been mostly demonstrated using middle-highly effective doses and rectal probe measurements after single oral dosing. In addition, CPM and DLM have been reported to produce biphasic dose-effect relationships: mild hyperthermic effects at low doses, and the expected hypothermia at greater dosages. At present, there is insufficient data to establish if the joint neurotoxicity of pyrethroids is influenced by the structure-specificity of the endpoint selected for study. In order to explore structure- and dose-related actions, we obtained time- and dose-effect relationships for above pyrethroids using mini-transponder technology (BMDS, USA). These telemetry-based chips collect and transmit real-time, subcutaneous body temperature (T) in a stressless fashion. Adult Wistar rats were implanted ~96 h before testing and TR was monitored to determine individual physiological baselines. The testing day, scans were taken at 30 min intervals for 5 hr after a single oral dose (~1-20% LD50; 1 ml/kg) of each compound dissolved in corn oil (N = 6-8 per dose group). The expected type I- and II-like TR patterns were observed at highly effective doses (in mg/kg: PM 150, BIF 12, DLM 9, α-CPM 15). However, a mild increase in T was observed for all pyrethroids at much lower doses regardless of structure. These preliminary results suggest that the dual, type I/II classification only may come into play above a certain threshold dose for a compound-specific toxicogenomic pathway. We plan to test the joint action of low-effective doses of all of these pyrethroids using TR as an endpoint to explore the impact of above findings on cumulative risk estimation.

PS 1347 ROLE OF DOPAMINE TRANSPORTER IN MANEB AND MANCOZEB TOXICITY.

S. Cheng, J. Quinones and C. Cuellar. *Sciences, John Jay College, New York, NY.*

Studies showed that pesticide exposures, such as dithiocarbamates, can potentiate the effects of the neurotoxin MPTP in dopaminergic neurons of mice eventually leading to neurodegeneration. Dopamine transporter (DAT) is a protein known to play a role in MPTP's toxicity by transporting MPP+, the metabolite of MPTP, into dopaminergic neurons. Alpha-synuclein, a DAT-interacting protein, has been known to aggregate into fibrillar cytoplasmic inclusions (Lewy bodies) which has been shown to be a key pathological marker associated with Parkinson's disease. One of alpha-synuclein functions is to mediate the recruitment or maintenance of DAT on the cell surface. Our preliminary data demonstrated that maneb (MB) and mancozeb (MZ), the Mn-containing dithiocarbamates, enhance MPP+-induced cell death in PC12 cells. However, the neurotoxic mechanisms involved in this action are still not clear. Our hypothesis is that MB and MZ increase the interaction of DAT and alpha-synuclein followed by increasing cell surface DAT expression which in turn enhances MPP+ cytotoxicity. PC12 cells were treated with MB (20 uM) and MZ (20 uM) for 1 h at 37°C/5% CO2. After treatments, the biotin-labelled cell surface proteins were isolated and subjected to Western blot analysis for evaluating changes in cell surface DAT expression. The data showed biotinylated DAT was increased (about 200%) after pesticide treatments. Total cell lysates were subjected for co-immunoprecipitation by anti-DAT antibody. Co-immunoprecipitated proteins were subjected to Western blot analysis for alpha-synuclein and DAT. The results showed the interactions of alpha-synuclein and DAT after pesticide treatments were increased about 200%. These results demonstrated that the expression of cell surface DAT and the interaction between DAT and alpha-synuclein are regulated by MB and MZ. Further experiments will be needed to elucidate the direct involvement of alpha-synuclein and dopamine transporter in the potentiated effect of dithiocarbamates in MPP+ induced neuronal cell death.

PS 1348 USE OF NON-BIASED STEREOLOGY TO ESTIMATE THE NUMBER OF TH+ NEURONS IN THE *SUBSTANTIA NIGRA* OF 8 AND 16 MONTH OLD MALE AND FEMALE C57BL/6 MICE REPEATEDLY EXPOSED TO PARAQUAT AND MANEB.

J. P. O'Callaghan[1], K. A. Kelly[1], D. B. Miller[1], R. C. Switzer[2], E. C. Lau[3], A. A. Li[3] and L. J. McIntosh[3]. *[1]Neurotoxicology Laboratories, Centers for Disease Control, Morgantown, WV, [2]NeuroScience Associates, Knoxville, TN and [3]Exponent, Inc., Menlo Park, CA.*

A proposed animal model for Parkinson's Disease is combined intraperitoneal exposure to paraquat (PQ) and maneb (MB) in C57Bl/6 mice. Stereologic methods were used to estimate tyrosine-hydroxylase positive (TH+) neuron number in the substantia nigra pars compacta (SNpc) of mice exposed to 3 dose levels of PQ+MB postnatally (PND) prior to weaning or both PND and as adults. Four groups of pups were dosed on PND 5-19 with 0/0, 0.3/1.0, 0.06/0.18, or 0.0007/0.0021 mg/kg PQ and MB (PQ/MB), respectively. These pups were divided into 3 sets of

mice, each with 4 dose groups. One set dosed only PND was sacrificed at 8 months of age. Two sets were dosed again as adults twice weekly during weeks 27 to 31 of age with 0/0, 10/30 or 5/15, 0.6/1.8, or 0.0007/0.0021 mg/kg PQ/MB. (The scheduled 10/30 dose was reduced to 5/15 after adult male mortality occurred in one group). One set of PQ+MB groups dosed PND and as adults was sacrificed at 8 months of age, the other at 16 months of age. Brains of all animals were serially sectioned at 50 uM thickness, immunostained for TH, and counterstained for Nissl using thionine. Every 6th section in the SNpc was counted using stereological techniques with Stereo Investigator (MicrobrightField, Inc. Williston, VT) v9.0 software to give a non-biased number of TH+ cells in the SNpc using the optical fractionator method. A treatment-related decrease in SNpc TH+ neuron count was measured in high dose males dosed as pups and as adults and sacrificed at 16 months of age (n=5-6; p=0.06 Tukey-Kramer). There were no differences from controls in TH+ neuron number in females sacrificed at 16 months. At completion of the study, the total number of animals per group will be 8-11.

PS 1349 PARAQUAT AND MANEB ALTER CELLULAR REDOX STATUS BY NON-REDUNDANT MECHANISMS IN SH-SY5Y NEUROBLASTOMA CELLS.

J. Roede and D. P. Jones. *Medicine, Emory University, Atlanta, GA.*

Parkinson's disease (PD) is the most common neurodegenerative movement disorder; however, less than 10% of all cases can be attributed to a genetic component. Therefore, the vast majority of PD cases are considered idiopathic and epidemiological studies have implicated chronic pesticide exposure as contributing to the etiology of PD. Recent epidemiological and in vivo evidence indicates that exposure to the pesticides paraquat (PQ) and maneb (MB) results in an increased risk of developing PD and dopaminergic cell loss, respectively. The purpose of this study was to investigate the mechanism of PQ and MB toxicity and evaluate how these agents alter cellular redox status and cause cell death in vitro. Using SH-SY5Y neurblastoma cells we found that MB was 25 times more toxic than PQ after 24 hours. PQ treatment caused a significant increase in intracellular ROS production; however, MB did not increase ROS production. Also, PQ treatment resulted in significant alterations in the redox state of the thioredoxin/peroxiredoxin system, while MB primarily altered cellular GSH redox. Taken together, PQ and MB alter cellular homeostasis is a non-redundant manner, which potentially exacerbates toxicity when cells are exposed to PQ and MB in combination.

PS 1350 MANEB (*MANGANOUS ETHYLENEBIS[DITHIOCARBAMATE]*) EXPOSURE IN RAT HIPPOCAMPAL ASTROCYTES LEADS TO ACTIVATION OF INTRINSIC APOPTOTIC PATHWAY.

M. Akhtar and L. D. Trombetta. *Pharmaceutical Sciences, St. John's University, Queens, NY.*

Maneb is a manganese-containing ethylenebis[dithiocarbamate] widely used fungicide. In general, dithiocarbamates have been shown to produce reactive oxygen species (ROS) and to alter cellular antioxidant homeostasis leading to oxidative stress. In addition, the exposure of pesticides may also be related to chronic disorders of the mammalian central nervous system (CNS). This relationship between dithiocarbamates and neurodegenerative disorders is increasingly being investigated. Previously, we have shown that, rat hippocampal astrocytes exposed to LC50 (13.5μM) concentration of maneb for 24 hours significantly increased lipid peroxidation. Moreover, significant alteration of cellular antioxidants level was also observed. The mode and mechanism of cell death in cultured rat hippocampal astrocytes due to maneb exposure is not yet elucidated. The purpose of this investigation is to elucidate the mechanism of cell death in rat hippocampal astrocytes treated with maneb. Rat hippocampal astrocytes were maintained in Dulbecco's modified Eagle's medium at 37°C, 8.0% CO_2 and supplemented with 10% FBS. Cells at 60-70% confluency were treated with 13.5 μM maneb. Caspase 3 & 7 activities were measured at 4, 8, 12, 16 and 24 hours of exposure. Caspase 9 activity was measured at 12, 16 and 24 hours of exposure. Cells were also harvested after 24 hours exposure for the analysis of cellular manganese metal concentration. Cells seeded in chamber slides were treated with Maneb 13.5 μM and after 24 hours of exposure the TUNEL assay was performed. Caspase 3 & 7 assay showed a significant increase ($P<0.05$) in caspase 3 & 7 activites at all time-points tested. In addition, caspase 9 activity was also significantly increased ($P<0.05$) at all time-points. TUNEL assay showed increased TUNEL-positive cells as compared to control. ICP-OES analysis of manganese cellular level showed a significant increase ($P<0.0001$) in manganese concentration in treated cells when compared to control.

PS 1351 THE NEUROTOXIC EFFECTS OF MANEB ON THE HIPPOCAMPUS OF NRF2 (-/-) MICE.

D. M. Kurzatkowksi and L. D. Trombetta. *Pharmaceutical Sciences, St. John's University, Queens, NY.*

Maneb is a ethylene(bis)dithiocarbamate fungicide that is used on a wide variety of crops, including, but not limited to fruit, nut, and vegetable crops, ornamental plants, and sod farms. Environmental exposure to this compound has been shown to cause alterations in oxidative defense mechanisms. This study investigated the neurotoxic effects of maneb on the hippocampus of C57BL/Nrf2 (-/-) knockout and C57BL/Nrf2 (+/+) wild type mice. Nrf2 (Nuclear factor-erythroid 2 related factor 2) is the prime transcription factor needed for the induction of a number of phase II detoxification genes through activation of the antioxidant response element (ARE). This knockout animal model is useful in showing the pro-oxidant effects of the metal-containing maneb. Intraperitoneal dosing took place twice a week for 30 days, at dosages 0, 15, 30, and 60 mg of maneb/kg body weight. Significant decreases in body weight were observed in the 60 mg/kg dosed Nrf2 (-/-) group as compared to the controls. Nrf2 (-/-) mice were also found to have an aberration of the hind limbs at the 30 and 60 mg/kg doses of maneb, suggesting hind limb paralysis. These animals dragged their hind limbs after 2 weeks of treatment. This phenomenon was never observed in the WT groups at any dose. Levels of total glutathione were significantly decreased in Nrf2 (-/-) mice as compared to Nrf2 (+/+) mice. These differences were observed at all treatment doses. Alterations in glutathione levels in the hippocampus and the observation of hind limb paralysis seen in Nrf2 (-/-) animals treated with the fungicide maneb, indicate increased susceptibility to neurotoxic injury. The lack of, or alterations to, the ARE complex are an indication of potential neurological insult and may have important environmental implications.

PS 1352 DEVELOPMENTAL EXPOSURE TO AMITRAZ ALTERS THE SEROTONIN SYSTEM.

M. R. Martínez-Larrañaga, J. Del Pino, M. A. Martínez, E. Ramos and A. Anadón. *Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Universidad Complutense, Madrid, Spain.*

Amitraz is a formamidine pesticide. The reported biological activity of the formamidines includes MAO inhibition, inhibition of prostaglandin synthesis, local anaesthetic effects and alpha-2 adrenergic activity. The continue presence of amitraz residues may be of particular concern to children and pregnant women. The objective of the present study was to investigate if perinatal amitraz exposure of rats during gestation and lactation alters serotoninergic neurochemistry in their offspring. Pregnant Wistar rats were orally gaved with amitraz (20 mg/kg) or with corn oil (1.0 ml/kg). The dosing period covered the period from day 6 of gestation through day-10 postnatal. Within each group, a total of 24 offsprings of different litters [12 of them control and 12 experimental (six males and six females)] were randomly culled on the lactation day 10. At 60 days of age, male and female offspring of control and amitraz-treated animals were killed and their brains removed. The frontal cortex and striatum were dissected and analyzed for content of serotonin (5-HT) and its metabolite 5-hydroxy-3-indole acetic acid (5-HIAA) using a HPLC method with electrochemical detection. Developmental amitraz exposure decreased 5-HT levels of male and female offspring at 60 days of age in the frontal cortex (17% $P<0.001$ and 30% $P<0.001$) and striatum (24% $P<0.01$ and 13% $P<0.05$) respect to corn oil controls. Developmental amitraz exposure also decreased 5-HIAA levels of male and female offspring at 60 days of age in the frontal cortex (22% $P<0.001$ and 8% $P<0.01$) and striatum (10% $P<0.001$ and 11% $P<0.05$) respect to corn oil controls. These results suggest there are long-term alterations in the serotonin system after developmental amitraz exposure. This work was supported by projects Ref. BSCHGR58/08(UCM), Ref.No S2009/AGR-1469(CAM) and Consolider-Ingenio 2010 No.CSD2007-063(MEC), Spain.

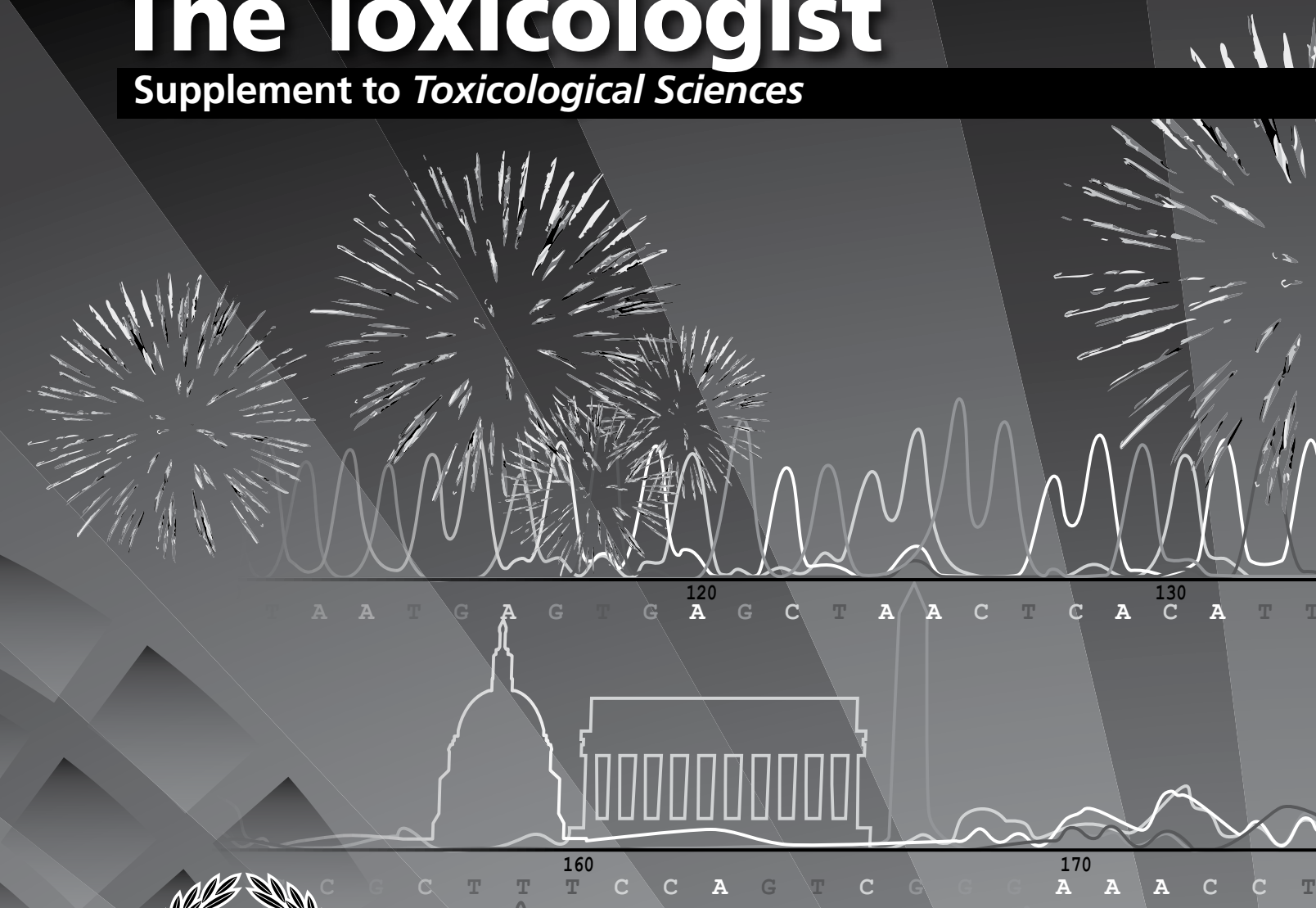
PS 1353 SODIUM CHANNEL BLOCKER INSECTICIDES (SCBIS) ARE STATE-DEPENDENT INHIBITORS OF MAMMALIAN VOLTAGE-GATED SODIUM CHANNELS.

R. T. von Stein and D. M. Soderlund. *Entomology, Cornell University, Geneva, NY.*

Sodium channel blocker insecticides (SCBIs) are a novel group of structurally diverse insect control agents that includes two compounds (indoxacarb and metaflumizone) registered for commercial use in the United States. SCBIs interact with voltage-gated Na+ channels in both insects and mammals and inhibit nerve function through a novel mode of action that is distinct from pyrethroid insecticides, which act elsewhere on the Na+ channel protein. Voltage-clamp analyses of Na+

The Toxicologist

Supplement to *Toxicological Sciences*



SOCIETY OF TOXICOLOGY
50th Anniversary
1961–2011

Celebrating 50 Years of Service in Science

50th Anniversary Annual Meeting and ToxExpo™ Washington, D.C.

An Official Journal of the Society of Toxicology

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 120, Supplement 2
March 2011

www.toxsci.oxfordjournals.org

SOT | Society of Toxicology

Creating a Safer and Healthier World by Advancing the Science of Toxicology

www.toxicology.org

Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 50th Annual Meeting of the Society of Toxicology, held at the Walter E. Washington Convention Center, March 6–10, 2011.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 578.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 606.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence.

Copies of *The Toxicologist* are available at $45 each plus $5 postage and handling (U.S. funds) from:

Society of Toxicology
1821 Michael Faraday Drive, Suite 300
Reston, VA 20190

www.toxicology.org