



- The Hebrew University Center of Excellence in Agriculture and Environmental Health •
- The Hebrew University of Jerusalem • October 2014 •

HU CEA EH – Year Two

Two academic years have elapsed since the establishment of the HU CEA EH. During this period we have witnessed the consolidation of the research and non-research activities of the Center. With the funding and aid of the EHF – Environment and Health Fund, our members have been involved in a variety of academic activities such as



international symposia and workshops which have greatly contributed to capacity building. Our members have also been involved in important reaching-out activities such as training of high school teachers of environmental science from all over the country.

In this newsletter we mainly highlight the achievements of the research teams and the students involved in the Center during the second year of activities. The completion of the project on exposure of children to pesticides via food consumption, the recruiting of additional students and the beginning of the work with healthy volunteers for the project on urinary levels of carbamazepine in healthy subjects exposed to crops irrigated with treated wastewater.

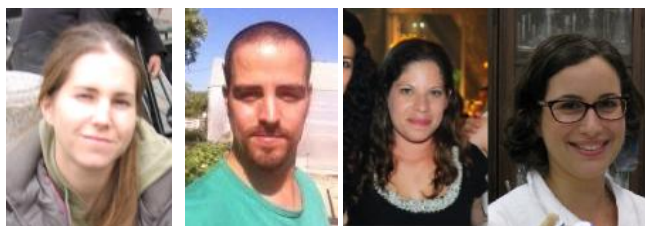
Welcome to Galit Tadmor, MSc

One of CEA EH main objectives is to promote capacity building in Environmental Health. We welcome **Ms. Galit Tadmor** who has recently joined the Center and is actively involved in two projects of the Center. Galit is helping in the development of analytic methods for measuring xenobiotics in plant matrices (fruits, leaves and roots) for project 1 and in human biological matrices for project 3. Galit finished her MSc in Water and Soil Sciences under the guidance of Prof. Benny Chefetz and was recently accepted by the Braun School of Public



Health as a MPH student under the guidance of Prof. Ora Paltiel. Her training represents a unique approach to capacity building within the Center. We welcome Galit and wish her lots of success in the MPH studies.

PhD Students and their achievements



Myah Goldstein, Tomer Malchi, Dorit Kalo and Naama Golan

Travel Awards

Three of our PhD students have been awarded travel grant by the **EHF** which will enable them to participate in the Young Researchers Conference on Environmental Epidemiology organized by ISEE-Europe. A main theme of the conference will be the “Exposome” but the conference will cover all areas of research in environmental epidemiology. The Conference will be held in Barcelona, Catalonia, Spain on 20 and 21 October 2014 at the Centre for Research in Environmental Epidemiology (CREAL). Congratulations for this achievement go to **Tomer Malchi, Dorit Kalo and Naama Golan**.

International Conferences, Seminars and Workshops

Gordon Research Conferences - Environmental Endocrine Disruptors: An Integrated Perspective from Wildlife to Human Health May 11-16, 2014 Renaissance Tuscany II Ciocco Resort Lucca (Barga), Italy

Dorit Kalo, participated in the highly prestigious Gordon Conference in Italy. A small group from our Center included two of our PIs, Dr. Ronit Calderon and Dr. Zvi Roth, met with Prof. Brenda Eskenazi—the chair of our SAC—who was also presenting at the conference.

EmCon 2014: 4th International Conference on Occurrence, Fate, Effects & Analysis of Emerging Contaminants in the Environment. University of Iowa – Iowa City, Iowa – August 19-22, 2014

Myah Goldstein and Naama Golan, participated in the EmCon Conference in Iowa. Both students presented posters. This conference provided a forum for presenting the latest research and development on all aspects related to the topic of emerging contaminants (ECs) in the environment. Prof. Benny Chefetz was also present at the conference.

Meetings like these present additional opportunities for capacity building within the Center.



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Projects Updates

Project: Urinary levels of carbamazepine in healthy subjects (Ora Paltiel, Yehoshua Maor, Galit Tadmor and Benny Chefetz)

This study represents a first effort to determine whether carbamazepine (used as a model for highly persistent pharmaceutical compound) can be detected in healthy individuals which were not exposed clinically to carbamazepine. During the month of September 2014 we performed the long awaited experiment with human volunteers. This part of the study was successfully completed. Volunteers from two campuses of the Hebrew University in Rehovot and Ein-Karem received explanation of study procedures and signed informed consent, participants were asked to eat over a 14 day period vegetables provided by farmers who grow their crops with reclaimed wastewater as irrigation water with approval from the Ministry of Health.



Thanks to the students of the Center who took part in the various parts of this project. From left to right Tomer Malchi helping us with the crops irrigated with treated wastewater, a volunteer answering the questionnaire and Galit Tadmor distributing the basket of produce to the volunteers in Rehovot.

As pictured above, a comprehensive food frequency questionnaire was administered at baseline and on the last day of the study. Analyses of the vegetables will also be conducted using established methods. Participants were asked to provide urine samples during the experiment which will be analyzed for the carbamazepine and its metabolites. The analysis of the biological specimens will follow.



From left to right: preparation of the samples for analysis by LC-MS; Distribution of the vegetables and water and checking the answers of the questionnaire to ensure compliance of the volunteers in Hadassah Medical School and preparation of the urine samples to be frozen before the analysis.

Project: Exposure of pregnant women and their offspring to EDCs and pesticides (Ronit Calderon, Ela Ein-Mor and Tamar Berman)

This project evaluates intrauterine exposures to phthalates, bisphenol A, Genistein, and organophosphates. How many subjects were recruited? How many samples were collected? With the increase in the number of births to women in our cohort, three neonatologists are now conducting the postnatal measurements and examinations. Furthermore, we have expanded the postnatal interviews to allow additional data collection, especially on mental health and exposure to stress during pregnancy, and to allow data harmonization with other studies conducted in Israel. Preliminary results were collected for 93 mothers and offspring whose data was entered. Offspring were born at an average gestational age of 38.9 (SD:1.9) weeks, to mothers with an average age of 31.8 (SD: 5.0) years. About three quarters of offspring were born in vaginal deliveries and about one quarter of the offspring (25.3%) were born in cesarean sections. Compared with all deliveries in Hadassah, 2004-2010, mothers in our cohort are slightly older (Hadassah mean: 29.8); however, gestational age and birth weight are similar, supporting the generalizability of our findings.



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Projects Updates

Project: Uptake of xenobiotics by crops

(Myah Goldstein, Tomer Malchi, Galit Tadmor, Yehoshua Maor, Naama Golan and Benny Chefetz)

We have conducted greenhouse and lysimeters plant-uptake experiments using different soils and wastewater qualities. The experiments were conducted with cucumber, tomato, carrot and sweet potato. The work was conducted as part of the PhD dissertation of Myah Goldstein and M.Sc. Thesis of Tomer Malchi. Major findings of this project indicate that the concentration of most pharmaceutical compounds in cucumber and tomato leaves were of similar order, but their concentrations in the tomato fruit were much lower than in the cucumber fruit. This suggests lower exposure potential when consuming tomatoes. Similarly, the concentrations of all studied pharmaceutical compounds were significantly higher in the leaves versus the roots of the root vegetables. These studies were the first to report the occurrence of carbamazepine metabolites crops. Our data suggest that metabolism of pharmaceutical compounds has to be evaluated to reveal the total uptake.



The health risk associated with consumption of wastewater-irrigated root vegetables was evaluated in this project.

Carbamazepine metabolites were found mainly in the leaves, where the concentration of the metabolite 10,11-epoxycarbamazepine was significantly higher than the parent compound.

The health risk associated with consumption of wastewater-irrigated root vegetables was estimated using the threshold of toxicological concern (TTC) approach. Our data show that the TTC value of lamotrigine can be reached for a child at a daily consumption of half a carrot (~60 g). This study highlights that certain PCs accumulated in edible organs at concentrations above the TTC value should be categorized as contaminants of emerging concern. Our data have been summarized in two papers (see publications list).

Project: Exposure of Israeli children to pesticides via food consumption (Orly Manor and Shirra Freeman)

This project was the first within the Center to be completed. The objective of this project was to assess uptake of agricultural pesticides through diet in a sample of Israeli children. An empirical modeling platform was populated with data of food consumption and on pesticide residues in order to estimate uptakes of 26 parent compounds 27 fruit and vegetable items. These were compared to the regulatory limits (Acceptable Daily Intake (ADI)) and to comparable results for adults. The results of this research project indicate that a total of 121 compounds were detected in the 18 selected food items. Twelve compounds were detected in >1/3 of the items. Samples with no residues detected were present in all 18 types. Two compounds, Methamidophos and Dimethoate exceeded ADIs for higher consumption quartiles. Children's uptakes (partial diet) were found to exceed those of adults (whole diet) in 12 compounds and were above the statutory limits in 10 items. The final results were presented in a poster at the ISEES meeting in August 2014 and as an oral presentation to the ISEE meeting in September 2014. An advanced draft of the paper is complete and submission is planned for October 2014.

Dr. Shirra Freeman, who was conducting this research project has left the Center and is currently the Lead Scientist at the Environmental Health Unit and Center for Excellence in Research on Environmental Health and Aging at Migal, Galilee Research Institute, Kiryat Shmona, Israel. We wish Shirra much success in her new endeavors.



Project: Pesticide exposure in adult male Jerusalem cohort (Hagai Levine and Jeremy D. Kark)

We finished laboratory analysis of organophosphate pesticides exposure in urine samples of 315 Israeli and Palestinian male residents of Jerusalem. We are currently conducting data analysis for assessment of magnitude and predictors of exposure. In parallel, we are conducting measurement of sex hormone levels in serum among the same individuals which will allow us to analyze associations between organophosphate levels and hormone levels.



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Project: Xenobiotics in dairy cows and their products (Zvi Roth and Dorit Kalo)

In this project, we aim to identify relationships between crops and contamination of dairy food products by using *in vitro* and *in vivo* animal models to clarify the risk associated with EDC exposure on both livestock and humans.

Samples of total mixed ration (TMR) and milk were taken from three farms that receive the diets from three commercial feeding centers. Samples were collected twice. We assume that TMR containing higher concentrations of xenobiotics, might also increase in the milk.

Samples were stored at -20°C and analysis of xenobiotics is underway. In addition, fresh crops used for dairy cow feeding were sampled from fields irrigated with wastewater (alfalfa) or rain water (vetch). Carbamazepine concentration in both alfalfa leaves and stems was approximately 1 ng/g. Unexpectedly, the concentration of carbamazepine in the vetch biomass irrigated with rain water ranged between 0.25 and 0.88 ng/g, and that of caffeine between 1.56 and 3.96 ng/g. These crops were used to prepare the TMR for growing calves. Samples were stored and an analysis for the presence of carbamazepine is under development. We plan to repeat the sampling in year 3 to verify the results.

We established an *in vivo* model to examine the uptake of di(2-ethylhexyl) phthalate (DEHP) via the rumen and its accumulation in adipose tissue, milk, plasma, urine, and follicular fluid. Milk, plasma and urine samples were collected and placed inside glass containers, and analyzed for the presence of DEHP and its metabolites. In general, the uptake and clearance kinetics of DEHP and its metabolites did not differ between milk, plasma and urine, characterized by a high level of phthalates absorbed from the rumen into the circulation, milk and urine. Clearance of all of the metabolites was relatively rapid, returning to basal levels within a few days. Analysis of the adipose tissue will be performed soon, with the aim of clarifying whether the reduction in phthalate concentration in the circulation is due to beneficial clearance or



Cows used in the *in vivo* model of this project.

accumulation in this tissue. A manuscript is being prepared with these results. Using an *in vitro* model using cow oocytes we have found that 50 µM bis(2-methoxyethyl) phthalate (MEHP), a phthalate metabolite, impaired both nuclear and cytoplasmic maturation. Moreover, MEHP increased the proportion of DNA-fragmented oocytes (i.e. TUNEL positive oocytes) in association with reduced mitochondrial function, increased Reactive Oxygen Species (ROS) and reduced mitochondrial membrane polarity. These findings might explain in part the reduced developmental competence of oocytes exposed to MEHP during maturation. An additional manuscript with these results is under preparation.

Capacity Building

Sabbatical - As of August 2014, **Dr. Hagai Levine** is on a one year Sabbatical (Funded by the Environment and Health Fund) at the Department of Preventive Medicine at the Icahn School of Medicine at Mount Sinai, New York. Dr. Levine is collaborating with **Prof. Shanna Swan** (a member of our center Scientific Advisory Committee), focusing on Environmental impact on Male Reproductive Health. His main project would be a meta-analysis of determinants of temporal and spatial variability in semen quality".



Prof. Shanna Swan and Dr. Hagai Levine pictured during the last SAC meeting in Israel (may 2013)

Coming up

CEAEH is planning to host the 2nd SAC (Scientific Advisory Committee) meeting on May 25-29, 2015. The meeting will be held in the Rehovot campus of the Hebrew University. An open conference will take place during the meeting.

Advanced Toxicology Course - In April 2014, the EHF funded the participation of **Dr. Yehoshua Maor** in the course Advanced Principles of Toxicology at the University of Guelph in Canada. Yehoshua spent two weeks in Guelph and received a thorough training in a wide range of subjects related to environmental toxicology such as mechanisms of toxic action, organic chemical contamination in soils and water, endocrine modulators and developmental & reproductive toxicology. Much of the course was dedicated to risk assessment. During his stay in Canada Yehoshua established contact with leading scholars in the field.

Publications

Goldstein M, Shenker M, Chefetz B. Insights into the uptake processes of wastewater-borne pharmaceuticals by vegetables. *Environ Sci Technol.* 2014;48(10):593-600.

<http://pubs.acs.org/doi/abs/10.1021/es5008615>

Malchi T, Maor Y, Tadmor G, Shenker M, Chefetz B. Irrigation of root vegetables with treated wastewater: evaluating uptake of pharmaceuticals and the associated human health risks. *Environ Sci Technol.* 2014;48(16):9325-33.

<http://pubs.acs.org/doi/abs/10.1021/es5017894>