Organic Pollutants in Environment – Markers and Biomarkers of Toxicity

Research Project
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Organic Pollutants in Environment – Markers and Biomarkers of Toxicity

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Project Holder

The Institute for Medical Research and Occupational Health (IMROH) was founded in 1947 at the location of Ksaverska cesta 2, Zagreb. Today, it is the second largest Croatian research institution under the Ministry of Science and Education. The areas of research interest IMROH covers and the professional services it provides include:

- Quality of working and living environment associated with the presence of different xenobiotics (pesticides, mycotoxins, heavy metals, radionuclides, nano-particles, drugs, etc.) and ionizing and non-ionizing radiation
- Cellular and molecular study of the impact of pollutants from the environment
- Development and treatment study of immunological and professional human diseases

The Institute's researchers participate in undergraduate and postgraduate university courses as well as in other professional studies and mentorships. IMROH publishes one of the oldest scientific journals in Croatia – *Archives of Industrial Hygiene and Toxicology*, with original articles from the field of occupational health, toxicology, ecology, chemistry, biology, biochemistry, pharmacology and psychology (http://hrcak.srce.hr/aiht?lang=en).

OPENTOX – Summary

OPENTOX *(Organic pollutants in environment – markers and biomarkers of toxicity)* is a project implemented by the Institute for Medical Research and Occupational Health and financed by the Croatian Science Foundation (HrZZ). It is based on Directive 2009/128/EC, the European Union Thematic Strategy on Sustainable Pesticide Use, the Collegium Ramazzini Statement, and the Stockholm Convention, all of which indicate the need to control excessive pesticide usage in order to mitigate their harmful effects on human health and the environment.

The environment is increasingly burdened by organic chemicals created as a consequence of anthropogenic activities.



Some are pesticides, i.e. agents used for pest control in the processes of food/feed production, storage and distribution.



The use of many pesticides was initially banned by EU Directives due to proven adverse effects and environmental accumulation. However, their large economic significance in certain member countries led to the approval of their restricted use in line with risk management. Subsequently, there has been a rise in the introduction of newer types of pesticides, developed as replacements for conventional pesticide types, but without sufficient scientific proof of safety towards non-target organisms, the toxicity of their metabolytes, as well as accumulation potential.



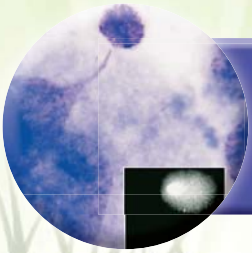
This strongly indicates the need for research into the mechanisms of toxicity and distribution of such pesticides in the environment and biosphere.

Our research carried out within five groups will focus on:

- Mechanism of genotoxicity of selected pesticides
- Oxidative potential of novel and conventional pesticides, as well as their mutual connection and connection with biomarkers of neurologic risks and carcinogenesis
- Cellular and subcellular toxicity of selected pesticides
- Effects on endocrine disruption of sex hormones and their connection to transplacental genotoxicity
- Distribution of selected pesticides and persistent organochlorine compounds in the biosphere

Research will be performed using four pesticide concentrations relevant to real scenario human exposure: occupational exposure limit (OEL), acceptable operator exposure level (AOEL), acceptable daily intake (ADI) and residential exposure level (REL).

The obtained insights are meant to significantly reduce the risk of residential exposure during pesticide handling and residing in the vicinity of treated agricultural fields. By implementing them into the Croatian National Plan on Sustainable Pesticide Usage and Polychlorinated Biphenyls Emission, they would be considered during the legislation of ecological food production, protection of soil, agricultural surfaces, and water.

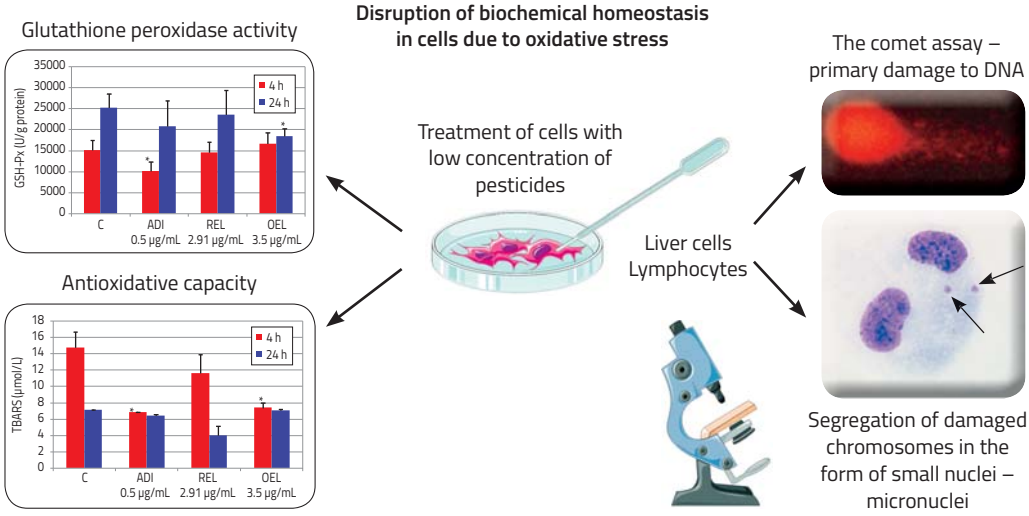


Genetic Toxicology of Pesticides

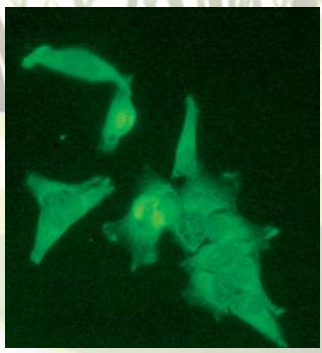
According to OECD guidelines, genotoxic tests were performed on different cell lines of human origin: human lymphocytes and human hepatocellular carcinoma cells with metabolic activation. Nevertheless, final testing was also done *in vivo* (encountering metabolisation of chemical in live organism). Cells were exposed to concentrations of pesticides reflecting real scenarios (ADI, REL, AOEL).

Results

Low doses of α-cypermethrin, chlorpyrifos and imidacloprid displayed DNA damaging potential, both in lymphocytes and liver-derived cells, inducing lymphocyte apoptosis (cell death), with higher cyto- and genotoxic effects of α-cypermethrin and chlorpyrifos. All three pesticides including glyphosate disturbed cell cycle kinetics, with tembotrione inhibiting enzyme 4-hydroxyphenyl-pyruvate-dioxygenase causing folate levels depletion, and glyphosate and α-cypermethrin causing probable crosslinking which in the case of glyphosate caused DNA damage in retardation. Our results demonstrated that the low permitted doses can have an impact on DNA stability as well as pointed to the possible inadequacy of animal and *in vitro* models and the time period for DNA damage assessment.

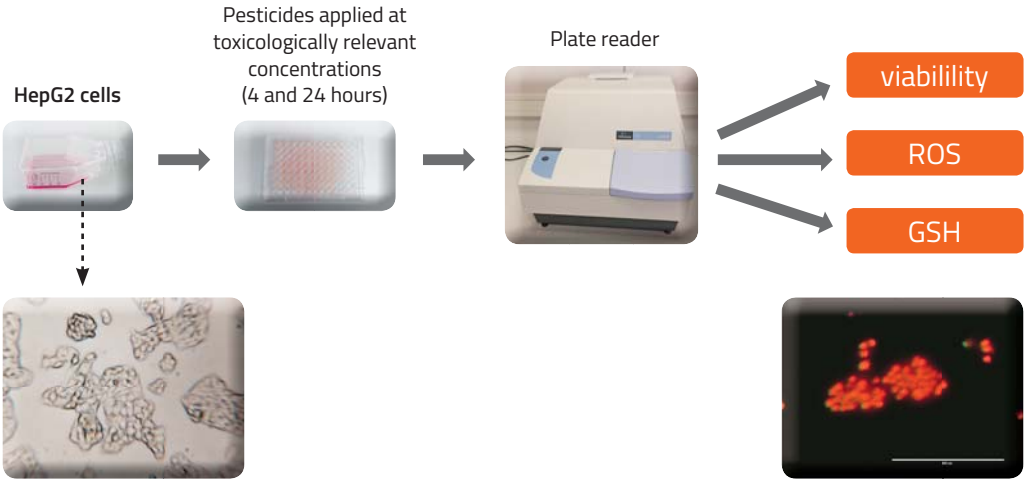


Cellular and Subcellular Toxicity



The toxicity of low pesticide concentrations that could be encountered in everyday life was tested using three insecticides: chlorpyrifos, imidacloprid, α-cypermethrin; and one herbicide: tembotrione. An *in vitro* study was conducted on the HepG2 human liver carcinoma cell line. The cells' ability to live (viability) was determined spectrophotometrically measuring the amount of coloured product formed as a result of cellular dehydrogenase activity in living cells. Significantly reduced cell viability was detected after 24 h exposure to α-cypermethrin at a concentration that corresponds to the OEL.

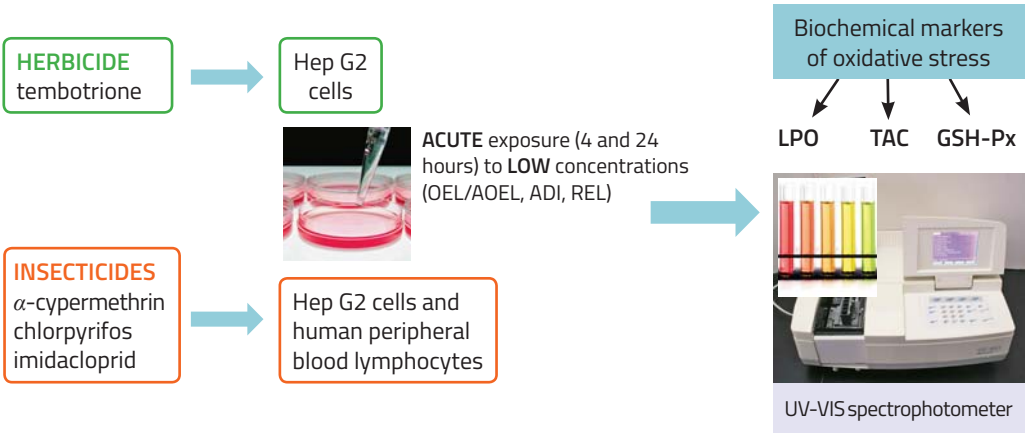
Impaired balance between level of reactive oxygen species (ROS) and cellular antioxidant defence could have also contributed to the pesticide's toxic effect. Levels of ROS and antioxidant glutathione (GSH) in the cells were measured after tembotrione exposure by fluorescent probes. After 24 h of exposure, a significantly higher level of ROS was observed for each pesticide concentration, while a reduced GSH level was detected at concentrations that correspond to the ADI and REL.



Biomarkers of Biochemical Toxicity and Oxidative Damage

Biochemical biomarkers of oxidative stress such as lipid peroxidation (LPO), total antioxidant capacity (TAC) and glutathione peroxidase (GSH-Px) activity were determined in human hepatocellular carcinoma cell line HepG2 after exposure to the herbicide tembotrione and in human peripheral blood lymphocytes and HepG2 cells after exposure to three insecticides: α-cypermethrin, chlorpyrifos and imidacloprid. The cells were treated acutely (4 and 24 hours) with concentrations relevant to real human exposure: OEL/AOEL, ADI and REL.

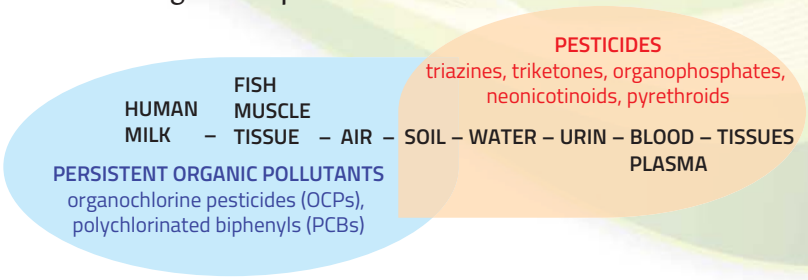
The end products of LPO were measured using thiobarbituric acid reactive substance assay with some modification. TAC was investigated using a FRAP assay that was slightly modified. The GSH-Px activity was measured by European standardized method.



The results showed that acute exposure of HepG2 cells to tembotrione as well as HepG2 cells and lymphocytes to all three of the insecticides did not cause alterations in any of the abovementioned biomarkers of oxidative stress.

Distribution of Pollutants in Environment

The project also includes a method development for the determination of the most frequently used herbicides and insecticides and persistent organic pollutants in the environment and biological samples.



Results of the determination of pesticides and organochlorine pollutants

AIR. The content of organochlorine contaminants in the air is temperature-dependent. Lower chlorinated congeners of PCBs and lindane are often present in urban air during warm periods, while the winter season air is represented by the combustion of process products, mostly HCB.

SOIL. Topsoil samples from the urban area often included indicator congeners of PCBs and pesticides: HCB, lindane, DDT and DDE. The levels of these compounds indicated their old input in the environment.

WATER. The concentrations of herbicides in tap and ground water determined within and near the area of Zagreb were below the legally set level. The pressure of agricultural activities on water was observed in surface water samples after herbicide application (June – August).

BIOLOGICAL SAMPLES. Low levels of organochlorine contaminants determined in fish muscle tissues originating from the Adriatic Sea showed no risk of chronic effects, but indicated an increased potential of bioaccumulation with age and body weight. PCB congeners: 153, 138, 180 and 170 and metabolite *p,p*-DDE were detected in human milk of multiparae in Croatia, but low levels of these compounds did not indicate a risk for breastfed infants. Analysis of 24-hour urine of rats during 28 days of repeated exposure via the oral route showed that more than 98 % of terbuthylazine was excreted as its metabolites. Mass fractions of excreted compounds were dose-dependent.