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The need for independent research: the contribution of the Ramazzini Institute

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Background. More than 200 compounds have been tested in long-term bioassays at the Cesare Maltoni Cancer Research Center (CMCRC), Ramazzini Institute (RI) and the results are used worldwide for hazard identification and human cancer risk assessments. Independence, consistency and quality of the methods used have been a distinguishing trait of the bioassays performed at CMCRC. A number of new challenges emerged in the last decade for toxicological research: 1) lack of funding for independent research 2) the need to evaluate hazards from low-doses of ubiquitous chemicals, as in the case of glyphosate-based herbicides (GBHs) 3) the need to consider multiple effects (e.g., cancer and noncancer) across multiple life stages 4) the need to reduce the overall number of animals 5) increased specialization and complexity of the studies that require collaborative efforts 6) the need to integrate evidence from toxicology with epidemiology and mechanistic evidence.

Methods/Approach. New funding strategies, innovative bioassays design and evidence-based methods have been developed by the CMCRC in order to face the challenges of modern toxicology. The Global Glyphosate Study of the RI represents a collaborative and paramount response to the challenges of modern toxicology.

Results.The RI already published the first results of the pilot phase of the Global Glyphosate Study that will be followed by an integrated experimental research project. The aim of the project is to explore comprehensively the effects of exposures to GBHs at current real-world levels on several toxicological endpoints. This study is independent of industry support and sponsored by a worldwide crowdfunding.

Conclusions. The RI was able to successfully move beyond cancer bioassays, while maintaining its standard of independence, consistency and quality of the methods. The Global Glyphosate Study represents an example of paradigm change in toxicology, based on the ever-ending value and need for independent research.

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Dr. Mandrioli is a physician and the Associate Director of the Cesare Maltoni Cancer Research Center, Ramazzini Institute. His work is focused on environmental and occupational in vivo toxicology, evidence-based methods and regulatory science.

The Ramazzini Institute 13-week study on glyphosate-based herbicides at human-equivalent dose in Sprague Dawley rats: first results and state of the art

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Background. Glyphosate-based herbicides (GBHs) are the most widely used pesticides worldwide, including the formulation known as Roundup. The massive and increasing use of GBHs raises concern of their possible effects on occupationally exposed individuals but also on general population. The current pilot study represents the first phase of a long-term investigation of GBHs that we are conducting over the next 5 years. In our pilot study, we evaluated the concentration of glyphosate and its major metabolite aminomethylphosphonic acid (AMPA) in urine, and we analyzed different end-points of toxicological and biological interest.

Methods/Approach.We treated Sprague-Dawley (SD) rats starting from prenatal life until adulthood with a dose of glyphosate equivalent to the United States Acceptable Daily Intake (US ADI) of 1.75 mg/kg bw/day, administered orally via drinking water. One cohort was continuously dosed until sexual maturity (6-week cohort) and the another cohort was continuously dosed until adulthood (13-week cohort). For each cohort, we evaluated urinary concentrations of glyphosate and AMPA, together with different parameters related to development, endocrine status and possible toxicological effect of GBHs.

Results. Both glyphosate and Roundup exposure led to comparable urinary concentrations of glyphosate and AMPA with an increasing amount of glyphosate found in urine in relation to the duration of treatment, indicating the systemic bioavailability of the active substance and a possible mechanism of bioaccumulaton. The concentration of both glyphosate and AMPA in the urine of SD rats treated with glyphosate was comparable to that observed in animals treated with Roundup, with an increase related to the duration of treatment.

Conclusions.The evaluation of different outcomes and endpoints of interest (i.e., pathology of target organs, molecular toxicity, genotoxicity, endocrine disrupting activities, microbiome, developmental toxicity, etc.) is currently ongoing in the different partner laboratories of the project.

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Dr. Panzacchi is head of the Unit of Data Management at the Cesare Maltoni Cancer Research Center of the Ramazzini Institute (CMCRC/RI). She obtained a degree in Biological Sciences and a Master in Biostatistic from the University of Bologna. She is involved in different in vivo projects at the CMCRC/RI and she presently is the Principal Investigator for the pilot study on Glyphosate and Roundup.

The Ramazzini Institute urges for new approaches in toxicology

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Background .Thousands of new synthetic chemicals have entered world markets in recent decades and are now beginning to be recognized as potential threats to health. Most chemicals have undergone little or no assessment of their safety or potential hazards to human health. To discover and quantify health effects associated with new and emerging chemicals have become priorities in toxicology.

Methods/Approach. First, we highlight that the current risk assessment for chemicals is industry-driven: most of the data are produced by companies and their contracted laboratories. We propose that safety testing be performed by independent laboratories, not commissioned by industry, but by a public authority. Furthermore, it remains to be clarified if current scientific methods are capable of addressing multiple effects (e.g., cancer and non-cancer) across life stages. We propose to adopt an innovative experimental approach (the Ramazzini Integrated Experimental Design – RIED) that integrates traditional cancer guidelines with more recent proposals of the OECD and US NTP for studying reproductive and developmental toxicity. The RIED methodology is aimed to increase the sensitivity of testing beyond that from commonly used protocols to give more reliable and inclusive information on many important endpoints in the context of whole-body physiology. The idea of commissioning independent peer-reviewed studies when there is controversy or scientific uncertainty is becoming one of the EU Commission's proposal to improve the transparency in risk assessment.

Results/Conclusions. The goal of toxicity testing is to develop data that can ensure appropriate protection of public health from the adverse effects of exposures to environmental agents. The proposed integrated protocol is ambitious and demanding in terms of resources. Nevertheless, such system could be considered as a sustainable chemical risk assessment on a long term perspective, with a considerable added value for policy-making. It's time to bring the toxicology into the real world.

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Dr. Manservisi is head of Special Project Units of the Ramazzini Institute; in this role she oversees programming, scheduling and conducting carcinogenicity bioassays for the identification of environmental toxins and carcinogenic agents. She is also involved in planning multiple-generation studies for the detection of endocrine interference activity for different chemicals.

Immunohistochemical characterization of lymphomas in a long-term rat study on Aspartame

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Background. The Ramazzini Institute (RI) has conducted three life-span bioassay studies of the carcinogenic effect of Aspartame (APM) administered with feed to rats and mice. Results indicated that APM is a multipotential carcinogenic agent. Particularly, the leukemogenic effect of APM was enhanced in rats when the exposure started from prenatal life, reaching statistically significant values in both male and female rats treated with 2000 ppm. In order to confirm and characterize the pathological diagnoses of lymphoma found in the RI study on the APM, we decided to perform the IHC analysis of all the cases of lymphoma, with particular attention on lymphoimmunoblastic type.

Methods/Approach. All the cases of lymphoimmunoblastic lymphomas diagnosed in the prenatal study on Aspartame (starting exposure from the 12th day of gestation) were studied by IHC. A panel of haemo-lympho-reticular tumors markers was selected, including PAX5, CD3, TdT and the proliferative marker Ki67. This same panel is routinely used for the differential diagnosis of lymphomas in humans. The IHC procedure was performed using an optimized protocol for alcohol-fixed paraffin embedded tissues based on our previous work.

Results. IHC analysis allowed us: 1) to characterize the type of haemo-lympho-reticular tumours based on the prevalence of B-cell (PAX5 expression), T-cell (CD3) lymphocyte or precursors cell lymphoblast (TdT) or reticular cells (CD68), 2) to characterize the lesions growth rate based on the degree of proliferation in the lesion (Ki67);

Conclusions. Our results confirm the usefulness of IHC for the characterization of haemolymphoreticular tumors, in particular lymphoimmunoblastic lymphomas. We aim to extend the IHC investigations implementing the use of other markers and analyzing tissues from other RI studies where increased incidences of lymphomas and leukemias are observed.

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Dr Tibaldi is a senior pathologist with the role of head of the pathology unit at the Cesare Maltoni Cancer Research Center, Ramazzini Institute. Her work is focused on pathology involved in screening slides to perform diagnosis and conducting peer review of histopathology for all types of in vivo studies.