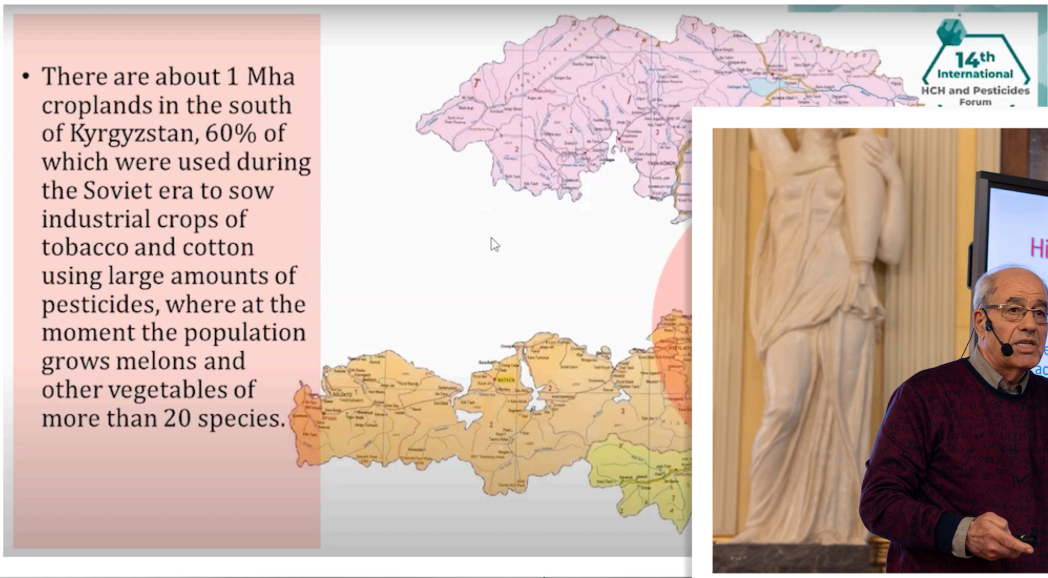
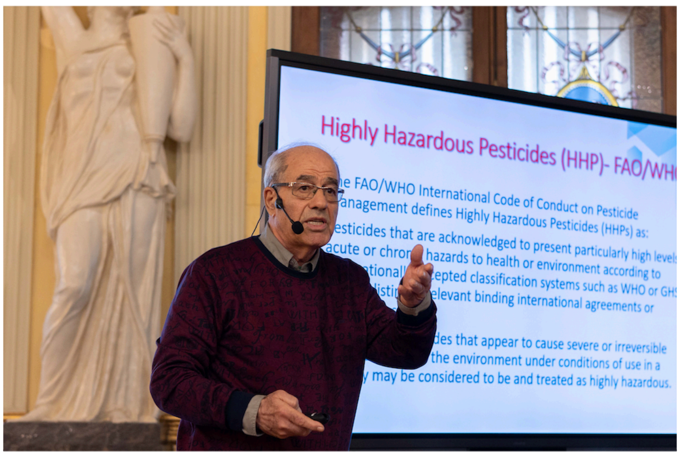
Block 14

TOXICOLOGY: CHEMICAL EXPOSURE OF WILDLIFE AND HUMANS





PESTICIDES AND WILDLIFE FRIENDLY FARMING

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Summary

Pesticides, agrochemicals used to protect humans from various diseases, are also known by their ability to cause a large number of negative health and environmental effects. We now know that some pesticides disrupt food webs, killing plants and insects and removing weed seeds from the environment, which may be essential food or habitat for some bird species and thus represents a high risk for their conservation.

On the other hand, we are living in a biodiversity crisis and there are multiple threats facing our wildlife, including habitat loss, pollution, and climate change. In order to tackle biodiversity crisis, we must look critically at every aspect of our lives, and see if what we are doing is compatible with nature.

SEO/BirdLife is very concerned by the environmental impacts of the widespread use of pesticides. We believe that if they must be used, it should be subjected to appropriate scrutiny, in a targeted way, and with respect to the environment. To do so, Governments should provide more support for farmers to reduce their reliance on chemicals, including the implementation of a national pesticide reduction target, encouraging the putting into practice schemes that assist farmers on improving the natural health of our countryside and providing independent advice and training to farmers.

The purpose of this conference paper is to set out whether our relationship with pesticides is safe for wildlife and the environment, and what we can urgently do to limit their harm.

Keywords

Pesticide, wildlife, farmland birds, biodiversity, impacts and management.

Introduction

Pesticides are agrochemicals used in agricultural lands, public health programs, and urban green areas in order to protect humans from various diseases. In agriculture there is no doubt that the emergence of chemical pesticides enabled an immediate intensification and increase of food production. However, due to their known ability to cause a large number of negative health and environmental effects, their side effects can be an important environmental health risk factor. In the 60 years since Rachel Carson highlighted the risks of pesticide use to wildlife and to people in her hugely influential book, *Silent Spring*, we have sleepwalked into a situation where pesticides have become the norm in terms of the way we manage land.

We are living in a biodiversity crisis, with around 1 million species facing extinction worldwide, populations of European farmland birds having decline by 4% since 1990 (Eurostat, 2021), and dramatic insect declines right across the globe.

For some specific pesticides, the negative impacts are well understood. But for many substances, huge gaps in our understanding of real-world impacts exist. However, we know enough to understand that the negative impacts of pesticides on wildlife are inevitable. Pesticides are rarely specific to a particular pest or weed, they persist in the environment for weeks, months or even years, they are rarely contained in the area they are applied and routinely contaminate nearby habitats. In addition, the cumulative effect of the sheer number of different chemicals, as well as the multitude of complicated ways wildlife can be impacted by exposure to chemicals, makes it difficult to quantify or understand this harm (RSPB, 2022).

Although it is important to use science to try and increase some of our understanding, instead of spending endless time and money trying to clarify every impact of every chemical on every species or ecosystem, we could realistically assume that chemicals designed to kill things, must be used minimally if we are to avoid damaging our world. We need a new approach to land management that focuses more on alternatives and less on chemicals, and ensures that those which are used cause minimal harm, are subjected to appropriate scrutiny, and are only used as a last resort. Many innovative and progressive farmers are working hard to reduce their pesticide use, but Governments need to step up their support for farmers to facilitate this at a larger scale and protect people and nature.

How much pesticide is used in Spain agriculture?

The vast majority of pesticide use in Spain is in agriculture. Most farms use a wide variety of different pesticides, most of which contain more than one active substance (the chemical within the product that is the active pesticide). Spain grows about 46.220.505ha of arable crops, but in 2019 (the most recent available data) the cumulative area treated with pesticides was around 48.053.966ha. The diagram below indicates the available data for overall treated area of arable crops in Spain and per type. The diagram shows that the number of ha treated with pesticides have increased by 4% in the last 8 years.

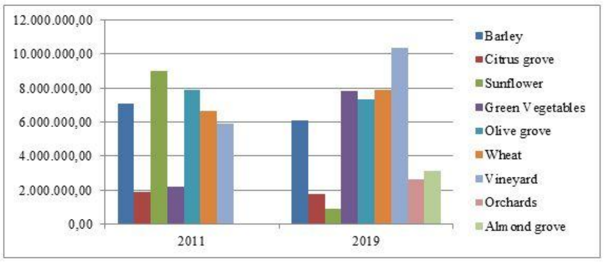

FIGURE 1. OVERALL TREATED AREA OF ARABLE CROPS IN SPAIN (2011 AND 2019). SOURCE: SPANISH STATISTICS INSTITUTE

The diagram below shows overall amount of pesticides per ha used in arable crops at a national level between 2011 and 2019. Data shows how use of pesticides per ha in Spain increased by 57% in the last 8 years.

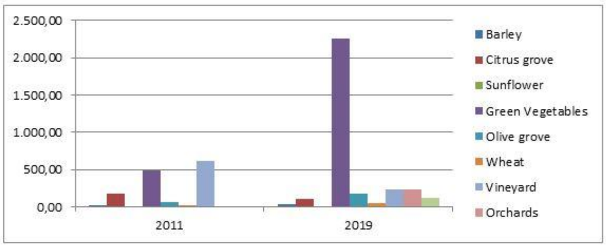

FIGURE 2. OVERALL KG OF PESTICIDES PER HA USED IN ARABLE CROPS IN SPAIN (2011 AND 2019). SOURCE: SPANISH STATISTICS INSTITUTE

Case study 1: Red legged and grey partridge

Pesticides disrupt food webs, killing plants and insects and removing weed seeds from the environment, which may be essential food or habitat for some bird species such as red legged partridge in Spain. Furthermore, the ingestion of cereal seeds treated with some pesticides reduces the reproductive success of this and other granivorous birds which represent a high risk for their conservation (Fernández V. *et al*, 2021; Lopez-Antia A., *et al*, 2021).

Previous studies on other partridge species (grey partridge) has shown that chick mortality was a key factor, and that this was related to the quantity of insects they had available to eat. An experiment was designed to test whether the herbicides and fungicides used on cereal fields reduce the survival of grey partridge chicks. The scientists discovered that the number of chicks in each brood halved when fields were sprayed conventionally, compared to fields where a 6m strip around the edge of the field was left unsprayed. Decreases in bird numbers were most rapid in areas that were most heavily polluted, suggesting that the environmental damage inflicted by these pesticides may be much broader than previously thought. Although this study looked only at the breeding success of grey partridge, it is highly likely that other insect eating farmland birds could be positively impacted in the same way when adequate management of pesticides is implemented (Rands, M. R. W., 1985).

Case study 2

Encouraging farmers to feel that they are part of the solution, not just the problem, is the first step in a long journey which will require financial and technical support, new partnerships and new visions, but this can hopefully be the start.

Martin is a Cambridgeshire-based arable farmer. His own farm is 160ha but including rented land and contracts, the total he manages is around 540 ha. Martin is passionate about working with nature across the whole farm to reduce the need for pesticides, and since 2013, has not used insecticides on his farm (RSPB, 2022).

Martin says there were some anxious moments when he first started, but profit margins have never suffered. He says that overall, on his farm, crop yields are a little lower, but the yield losses are more than made up for by the reduction in how much he spends to produce the food, and his farm is now more resilient to weather and market fluctuations. Martin is following an integrated pest management approach, using chicken manure to help improve soil and replace artificial fertiliser, and cover cropping and direct drilling.

He is Chair of the Nature Friendly Farming Network (NFFN, an independent organisation that encourages farmers improving the natural health of the countryside), he says that talking to other like-minded farmers as part of the NFFN has given him the opportunity to share best practice and understand that the best way we can farm is farming with our environment around us.

Conclusions

SEO/BirdLife is very concerned by the environmental impacts of the widespread use of pesticides. We believe that if they must be used, it should be with care, in a targeted way, and with respect to the environment. Our vision for agriculture is for sustainable systems of farming that produce safe, healthy food; safeguard our soil, air and water; and help to protect and enhance wildlife and its habitats.

Conclusion 1

SEO/BirdLife, along with other environmental, health and farming organisations, are calling for a national pesticide reduction target to be implemented in Spain. This has already happened in other European countries (eg. Denmark), and has shown to have a significant impact. A national reduction target will ensure that the most harmful pesticides to the environment or human health are prioritised for reduction. Meanwhile, a target for cutting overall use will ensure that indirect and poorly understood effects from pesticides are reduced.

Conclusion 2

SEO/BirdLife is calling on our Governments to provide more support for farmers to reduce their reliance on chemicals. It is vital that farmers are supported to do this through a variety of means, including the new land management schemes in each county, provision of independent advice and training, and increased research into alternatives (especially agroecological farming systems).

This should include the creation of an independent organisation (eg. Farming for Nature in Ireland and Nature Friendly Farming Network in the UK) that encourages farmers improving the natural health of our countryside. This organisation should be implemented with the help of environmental NGOs such as SEO/BirdLife, and should promote initiatives such as free training systems, gathering events (including forums, training courses, annual gatherings, etc), and resources. The initiative should include easily accessible information for all farmers in Spanish language.

Conclusion 3

Clearly, we cannot expect all farmers or land managers to cut out or dramatically reduce chemical pesticides overnight; many are tied into a system which is dependent on chemical use. However, with the right support and advice, changes can be made that will reduce reliance on pesticides over time and maintain profitable businesses and healthy yields. These include better support for an Integrated Pest Management (IPM) approach – where pesticides are used only as a last resource. This can include better monitoring of pests and diseases, more diversity in terms of which crops to grow and where, and creating habitats for nature to act as natural pest control, especially for those farmers located on Natura 2000 of high importance habitats. All information on IPM systems should be free and easily accessible for all farmers.

General principles of Integrated Pest Management (included on DIRECTIVE 2009/128/EC Annex III) should be followed by all business. To make sure that this is carried out we recommend Governments undertaking official controls carried out by the competent authorities to verify business compliance with the requirements.

Conclusion 4

For the sustainable intensification of the agricultural production is important to maintain a high productivity while protecting farmers, consumers and the ecosystem from the adverse effects of pesticides, a more sustainable way of increasing food production needs to be targeted.

In this regard, pesticide registration is an important step in the management of pesticides since it enables authorities primarily to determine which products are permitted to be used and for what purposes, and it also makes possible the control over quality, use levels, claims, labelling, packaging, and advertising of pesticides.

In this context, the proper evaluation of pesticides before they are registered for use is an important mechanism to ensure that only appropriate pesticides enter the national market. This is even more so considering that post-registration awareness building, training, compliance monitoring, and enforcement is relatively feeble in many developing countries.

Conclusion 5

We must recognise the important role that healthy thriving ecosystems can play in supporting sustainable farming. Pest predators, pollinators and wildlife in the soil can all help to grow food and sustain longterm yields, while continued reliance on pesticides threatens long term sustainable food production and a healthy environment. We must move away from our current situation, which focuses on an everincreasing reliance on chemicals to one which strikes a better balance with nature. We, and nature, depend on it.

Highly-contaminated areas where environmental impacts on nature and human health are already visible (eg Mar Menor in Spain) should be prioritised when supporting sustainable farming, with previously designed specific programs. In order to identifying these areas, SIGPAC Geographic Information System could be used.

Environmental stewardship refers to the responsible use and protection of the natural environment through active participation in conservation efforts and sustainable practices by individuals, small groups, non-profit organizations, federal agencies, and other collective networks. Encouraging environmental stewardship agreements with the main aim of reducing the risk and use of pesticides on the environment would be an effective way to supporting sustainable farming.

References

Eurostat: https://ec.europa.eu/eurostat/web/products-eurostat-news/-/edn-20210522-1

Fernández Vizcaíno, E., I.G. Fernández de Mera, F. Mougeot, R. Mateo y M.E. Ortiz Santaliestra. 2020. Multi-level analysis of exposure to triazole fungicides through treated seed ingestion in the red-legged partridge. Environmental Research, 189: 109928.

Lopez-Antia, A., M.E. Ortiz Santaliestra, F. Mougeot, P. Camarero y R. Mateo. 2021. Birds feeding on tebuconazole treated seeds have reduced breeding output. Environmental Pollution, 271: 116292

Rands, M. R. W. 1985. Pesticide Use on Cereals and the Survival of Grey Partridge Chicks: A Field Experiment. Journal of Applied Ecology, 22: 49-54

RSPB, 2022. Pesticides and wildlife: a decades old nature crisis.

TOXICOLOGICAL EVALUATIONS OF GLYPHOSATE IN ZEBRAFISH EARLY-LIFE STAGES

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Summary

The glyphosate is now considered the most widely used herbicide in the world. It is a systemic, non-selective, post-emergency herbicide. Its use in agriculture determines the destruction of unwanted weeds. Its mechanism of action is to block the enzyme 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS) which is involved in the shikimic acid pathway. The high persistence and its widespread determine the presence of traces of this herbicide increasingly frequently in soil, water and air. The present study aimed to evaluate the glyphosate potential effects on the development of zebrafish early-life stages. The research focused on the acute toxicity evaluation of glyphosate, by the Fish Embryo Acute Toxicity (FET) tests. Tested concentrations were 25 mg/L, 50 mg/L, 100 mg/L and 125 mg/L. Zebrafish larvae exposed to glyphosate showed mortality and developed sublethal alterations including yolk sac and pericardial edema, blood stasis, impaired blood flow and alterations in head development, suggesting a neurological involvement. The same sublethal alterations have been confirmed at histological level mainly including cardiac dilation, edema and head deformations. These results confirmed the developmental toxicity of glyphosate in zebrafish early stages then, the aim of future studies is to evaluate the long-term effects of this herbicide.
The project PEACH (RF-2016-02364628) is funded by the Italian Ministry of Health.

Keywords

Glyphosate; zebrafish early-life stages; FET Test; toxicity; sublethal effects; histological analyses

Introduction

Glyphosate (*N-(*phosphonomethyl) glycine) is a systemic and non-selective post emergence foliar herbicide. It is today known as the most widely used herbicide worldwide (O Duke & B Powles, 2008). Glyphosate operates via 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme. The EPSPS role is to catalyse the condensation reaction between shikimate-3-phosphate (S3P) and the phosphoenolpyruvate (PEP) resulting in 5-enolpyruvylshikimate-3-phosphate (EPSP). The glyphospate bonds to the EPSPS catalytic site, thus competing with the PEP. As a consequence, it creates a ternary complex glyphosate-EPSPS-S3P, which blocks the metabolic pathway and results in shikimate stack inside the cells (Duke, 2021). Indeed, although shikimate pathway is not found in humans, it is typical in bacteria, including those in the human gut microbiota (Mesnage et al., 2021). In addition, glyphosate and glyphosate-based herbicides can also be toxic for aquatic organisms. Ecotoxicology studies based on water organisms as experimental models are particularly useful, as glyphosate-based herbicides and their metabolites are often found in rivers and other water ecosystems (Gasnier et al., 2009) as result of rains and land erosion (Meftaul et al., 2020). Because the glyphosate is the most adopted herbicide worldwide (O Duke & B Powles, 2008), the present study aimed to investigate the glyphosate potential effects on the development of zebrafish early-life stages. The use of the zebrafish model is due to its advantages in behavioral research, molecular biology, developmental biology, genetics, toxicology, drug discovery, oncology, and neuroscience and also because it is an excellent tool to study the mechanisms of pesticide toxicity and the alterations they induce in aquatic organisms (Petrovici et al., 2020) while glyphosate was selected because of its wide use as herbicide in pre and post emergence and for its documented presence in water bodies.

Materials and method

Zebrafish early-life stages used in the experiments were obtained from the University of Teramo facility. Adult wild type AB strain zebrafish were breed in a recirculating water system with a light/dark cycle (14 hours of light/10 hours of dark).

The afternoon before the spawning, groups of females and males of adult zebrafish were placed in breeding tanks and the eggs were collected the following morning and rinsed by diluition water (DW). After the macroscopic selection (the unfertilized eggs were eliminated) the eggs were transferred to Petri dishes with DW.

Fish embryo acute (FET) toxicity tests

FET tests were performed according to OECD n. 236 (OECD, 2013). Glyphosate (Gliphogan Top CL PFnPE; Bayer Agriculture BVBA, B-2040 Antwerp Belgio) was tested at 25, 50, 100, 110 and 125 mg/L. These concentrations were chosen from previous dose ranging-finding tests. Glyphosate was dissolved in bidistilled water. Selected embryos were placed individually with 2 mL of test solution in each well of 24-well plates within 3 hours post fertilization (hpf). Twenty embryos for treatment were exposed to the four concentrations of glyphosate, and the working solutions were freshly prepared every 24 h. Negative control and positive control (4% 3,4 dichloroaniline) were also tested. Three replicates were performed. Embryos were exposed for 96 h in the incubator at 26 ± 1° C and photoperiod (14 h light/10 dark) conditions. Zebrafish early-life stages were daily observed up to 96 h with the inverted optical microscope considering four lethal alterations: coagulation of fertilized eggs, absence of heartbeat lack of somite formation, lack of detachment of the tail from the yolk sac and lack of somite formation. At the end of the exposure period, lethal concentration 10, 20, and 50 (LC 10, 20, 50) and some sublethal effects were evaluated.

Histological analyses

At 96 hpf survived larvae, treated with the following concentrations of glyphosate: 25, 50, 75, 100, and 110 mg/L, were collected for histological analyses. Embryos were fixed in 10% neutral-buffered formalin for 2h and processed for routinely histological evaluation. 5 µm thickness sections were stained with hematoxylin and eosin and then visualized by light microscope.

Statistical analysis

Statistical analysis of FET tests results was performed using ToxRat software version 3.3 (ToxRat Solutions GmbH, Germany).

Results

Fish embryo acute toxicity (FET) tests

The toxicity of glyphosate was determined at 96 hpf as lethal concentration, and LC10, LC20, and LC50 values were 85.7, 97, 122.9 mg/L, respectively. Late coagulation was the only lethal endpoint at the highest concentration of glyphosate (125 mg/L). Furthermore, zebrafish larvae treated with glyphosate developed sublethal alterations. From the concentration of 100 mg/L at 48 hpf, the larvae showed the yolk sac edema, pericardial edema, impaired blood flow and blood stasis (Figure 1). In addition, a great number of zebrafish larvae showed a smaller head then the negative control (NK), suggesting a possible neurological involvement (Figure 1). The number of survived embryos developing sublethal alterations increased in a concentration-dependent manner and in a time-dependent manner (from 72 hpf to 96 hpf).

Histological analyses

Histological observation showed the lack of morphological alterations in controls and in glyphosate 25 mg/L – treated embryos. Low pericardial edema was present in 50% of glyphosate 50 mg/L and 75 mg/L – treated embryos together with scattered liver macrovacuoles and dark hepatocytes. More evident alternations have been determined by higher glyphosate concentrations exposure: 80% of embryos treated with glyphosate 100 mg/ L showed high pericardial edema (Figure 3a) and diffuse hepatic microvacuoles (Figure 3b). Dimensional modification has been observed also in the brain. Finally, after 110 mg/ L exposure, 100% of survived embryos presented very high morphological alterations with lack of development and autolysis phenomena.

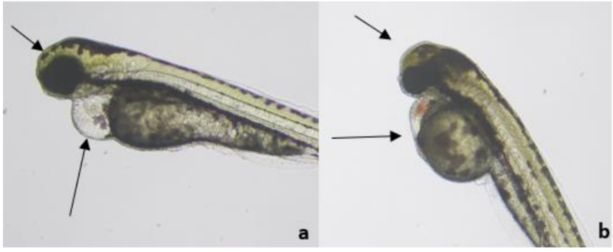

FIGURE 1. A) REPRESENTATIVE IMAGE OF PERICARDIAL EDEMA IN ZEBRAFISH LARVAE EXPOSED TO 100 MG/L OF GLYPHOSATE AT 72 HPF; B) REPRESENTATIVE IMAGE OF SMALL HEAD IN ZEBRAFISH LARVAE EXPOSED TO 100 MG/L OF GLYPHOSATE AT 72 HPF

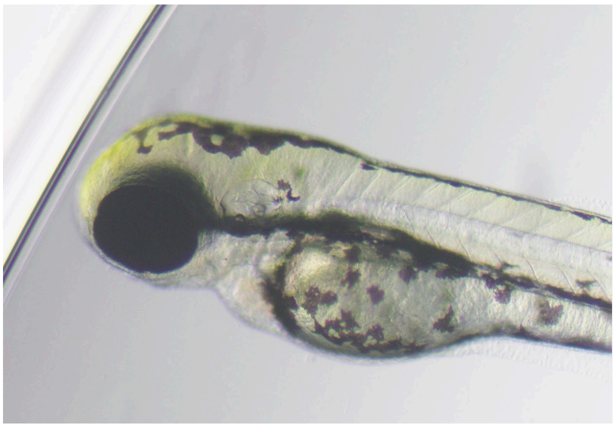

FIGURE 2. REPRESENTATIVE IMAGE OF THE PERICARDIAL REGION AND THE HEAD IN ZEBRAFISH LARVAE EXPOSED TO NEGATIVE CONTROL AT 72HPF

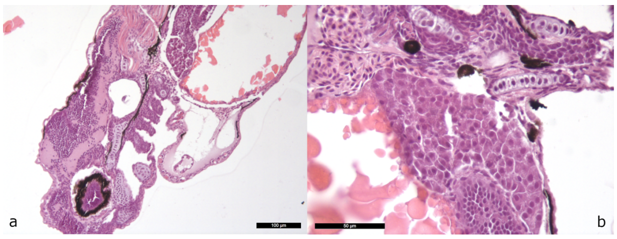

FIGURE 3. HISTOLOGICAL ALTERATIONS OBSERVED IN GLYPHOSATE 100 MG/ L TREATED - EMBRYOS SUCH AS INTENSE PERICARDIAL EDEMA (A) AND LIVER MICROVACUOLES (B)

Discussion

The extensive use of glyphosate in agriculture, traces of this herbicide are nowadays found in soil, water, and air (Krüger et al., 2014) as well as in food (Zoller et al., 2018), becoming a growing concern for human health.

Zebrafish larvae exposed to glyphosate developed also sublethal alterations including edema of the yolk sac, pericardial edema, impaired blood flow and blood stasis, in according with Sulukan et al., (2017). In the present study, as also demonstrated by Diaz-Martin et al., (2021) the developmental toxicity of glyphosate increased in concentration-dependent manner.

The cardiac alterations induced by glyphosate exposure in our study (blood stasis, impaired blood flow, and pericardial edema) can be related to dysregulation of transcription factors belonging to the family of myocyte enhancer factor-2 (MEF2), involved in the development of the heart muscle, as hypothesized by Roy et al., (2016). The same sublethal alterations have been confirmed at histological level mainly including cardiac dilation and edema. In this study the zebrafish larvae exposed to glyphosate, showed an altered morphology of the head with smaller heads than the negative control. This alteration may be caused by glyphosate-induced neurotoxicity: the glyphosate determines, during the embryonic stage, a reduction in the expression of essential genes for the normal development of the central nervous system, such as pax2, pax6, Otx2, ephA4, as demonstrated by Roy et Al., (2016). Furthermore, histological observation has allowed us to establish the presence of glyphosate dependent liver toxicity, previously observed in adult zebrafish (Giommi et al.,2022) and other species (Ren et al., 2019).

Conclusion

The results of the present study confirmed the developmental toxicity of glyphosate in zebrafish's early stages. Moreover, even its use in agriculture has been proclaimed safe because humans and other animals do not have the target EPSPS enzyme, however, increasing numbers of studies have demonstrated risks to humans and animals because the shikimate metabolic pathway is present in many microbes, and several studies demonstrate that more than one-half of human microbiome are intrinsically sensitive to glyphosate. Finally, the aim of future studies will be to evaluate the long-term effects of this herbicide.

References

Díaz-Martín, R. D., Valencia-Hernández, J. D., Betancourt-Lozano, M., & Yáñez-Rivera, B. (2021). Changes in microtubule stability in zebrafish (Danio rerio) embryos after glyphosate exposure. Heliyon, 7(1). https://doi.org/10.1016/j.heliyon.2021.e06027

Duke, S. O. (2021). Glyphosate: Uses Other Than in Glyphosate-Resistant Crops, Mode of Action, Degradation in Plants, and Effects on Non-target Plants and Agricultural Microbes. Reviews of Environmental Contamination and Toxicology, 255, 1–65. https://doi.org/10.1007/398_2020_53

Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M. C., & Séralini, G. E. (2009). Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology, 262(3), 184–191. https://doi.org/10.1016/j.tox.2009.06.006

Giommi C., Ladisa C., Carnevali O., Maradonna F., & Habibi HR. (2022). Metabolomic and Transcript Analysis Revealed a Sex-Specific Effect of Glyphosate in Zebrafish Liver. International Journal of Molecular Sciences, 23(5):2724. doi: 10.3390/ijms23052724.

Krüger, M., Schledorn, P., Schrödl, W., Hoppe, H., Lutz, W., & Shehata, A. (2014). Detection of Glyphosate Residues in Animals and Humans. Journal of Environmental & Analytical Toxicology, 4:2. https://doi:10.4172/2161-0525.1000210

Mesnage, R., Calatayud, M., Duysburgh, C., Marzorati, M., & Michael, N. (2021). Alterations in human gut microbiome composition and metabolism after exposure to glyphosate and Roundup and / or a spore-based formulation using the SHIME ® technology. Gut Microbiome, 3, E6. https://doi:10.1017/gmb.2022.5

O Duke, S., & B Powles, S. (2008). Glyphosate: a once-in-a-century herbicide. Pest Management Science, 63(11), 1100–1106. https://doi.org/10.1002/ps

Petrovici, A., Strungaru, S., Nicoara, M., Andreea Robea, M., Solcan, C., & Faggio, C. (2020). Toxicity of Deltamethrin to Zebrafish Gonads Revealed by Cellular Biomarkers. Journal of Marine Science and Engineering, 8(2), 73, https://doi.org/103390/jmse8020073

Ren, X., Dai, P., Perveen, A., Tang, Q., Zhao, L., Jia, X., Li, Y., & Li, C. (2019). Effects of chronic glyphosate exposure to pregnant mice on hepatic lipid metabolism in offspring. Environmental Pollution, 254. https://doi.org/10.1016/j.envpol.2019.07.074

Roy, N. M., Carneiro, B., & Ochs, J. (2016). Glyphosate induces neurotoxicity in zebrafish. Environmental Toxicology and Pharmacology, 42, 45–54. https://doi.org/10.1016/j.etap.2016.01.003

Sulukan E., Köktürk M., Ceylan H., Beydemir Ş., Işik M., Atamanalp M., & Ceyhun S.B. (2017). An approach to clarify the effect mechanism of glyphosate on body malformations during embryonic development of zebrafish (Daino rerio). Chemosphere, 180:77-85. doi: 10.1016/j.chemosphere.2017.04.018.

Zoller, O., Rhyn, P., Rupp, H., Zarn, J.A., & Geiser, C. (2018). Glyphosate residues in Swiss market foods: monitoring and risk evaluation. Food Additives and Contaminants: Part B Surveillance, 11(2), 83–91. https://doi.org/10.1080/19393210.2017.1419509

HIGHLY HAZARDOUS PESTICIDES (HHP) IN EU COMPARED TO DEVELOPING COUNTRIES, CASE STUDY: HHP HISTORY AND USE IN IRAN

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Summary

Highly Hazardous Pesticides (HHPs) in EU

Highly Hazardous Pesticides (HHP) are groups of pesticides with very high toxicity for man and wildlife, banned in most developed world countries but unfortunately still in use in some developing countries legally or illegally. HHPs were responsible for some world disasters already.

EU Bans Pesticides in Parks, Playgrounds, and Playing Fields. Highly Hazardous Pesticides (HHPs) nearly banned in all EU countries are still found easily in some developing countries doing tremendous harm to people, environment, and wildlife. Mass killing of people in Bhopal India, Iran, etc. mass killing of children in Peru a few decades ago and about 10 years ago in Bihar Province of India both due to eating HHPs polluted food are some examples at the time that big pesticide corporations just thinking about their own profits.

According to UNEP report: In 2015, SAICM Fourth International Conference of Chemicals Management (ICCM4) adopted a resolution that recognizes HHPs as an issue of international concern and calls for concerted action to address HHPs.

Highly Hazardous Pesticides (HHPs) History and use in Iran

According to my experience in the field and lab, in Iran like many other developing countries there are many people killed by pesticides despite our decades long activities to stop it. Biggest obstacle is lack of cooperation between different sectors and people for enforcement issues. HHPs are taking lives in many developing countries including Iran while pesticide corporations are making good profits with no care about the tragical -situation that HHP are creating in developing countries.

Death scenarios with HHP Ops in Iran:

Historically, highly toxic Persistent Organic Pollutants (POPs) plus HHPs Organophosphorus insecticides (Ops) like parathion, etc. in cotton fields, other agricultural fields and citrus orchards of Mazandaran and Golestan Provinces. HHPs like Parathion, Diazinon, Sevin, Paraquat already resulted in some mass killing in Iran.

Effects of HHP on wildlife, honeybees and pollinators:

HHPs are known to be prominent drivers of pollinator decline with long term risk for pollinators. Now bits and pieces of pesticides/ chemicals and their metabolites are found in all niches and habitats preventing the natural processes of speciation responsible for the development and diversification of animals since 100s millions of years ago. A pesticide is designed to kill, when pesticide molecules and later their metabolites enter agroecosystems, they are against the whole biodiversity.

Wildlife poisoning is a big problem everywhere, particularly in developing countries. Overuse and misuse of agrochemicals particularly HHPs and POPs during the past 100 years destroyed agricultural biodiversity in many developing countries due to lack of regulations and enforcement. Handling toxic compounds like pesticides in developing countries have been under lots of discussions due to lack of proper methods, infrastructures, etc., (how pesticides are handled in developing countries, my message more than 20 years ago). I am a senior pesticide toxicologist with 3-4 decades of experience in Iran and globally about the situation of pesticides regulations and enforcement in developing countries. There are big differences and gaps for toxic pollution reductions and regulations between Northern and Southern countries, industries in developed countries but more sufferings in developing countries.

Keywords

Highly Hazardous Pesticides, HCH, Enforcement, Wildlife, Developing countries.

Introduction

Being involved in pesticide toxicity testing since my BSc and MSc studies more than 40 years ago, I finally wrote a Probit analysis based computerized program for correct and fast LC50s, LD50s, etc. calculation during my PhD studies in the University of Guelph [1, 55, 56, 57, 58]. This was only possible to do with help of one of my professors: professor J.J. Hubert (Department of Mathematics and Statistics, University of Guelph) author of the book: bioassay, I am honored my project is referenced in the book [2]. I was always interested in correctly measuring the pesticide acute toxicities on different animals usually by conducting replicated bioassays. So, it was natural as a pesticide toxicologist to be very interested in dangerous compounds like HHPs of course to be able to better helping people, environment and wildlife against HHPs, the life policy that I have been following since my MSc research, please see later in this paper why? Fortunately, in recent years we discussed HHPs under many SAICM, BRS, other pesticide/ chemicals related conventions, discussion groups, conferences, etc., widely, and globally. I discussed how these compounds mostly as insecticides affect wildlife in Toledo Spain (2017) [3] by invitation from the UNEP-CMS preventing poisoning group. According to the WHO acute toxicity makes it possible to classify pesticides into five classes: class I - highly toxic, class II - toxic, class III - moderately toxic, class IV - slightly toxic, class V - virtually non-toxic.

Increasing pesticide use and loss of biodiversity on the planet: Now it is a decade that I myself as an entomologist always am worried about loss of biodiversity, am observing collapse in insect populations in many insect orders [53]. There are about 3 million species of insects on the planet, they are a very important part of the biodiversity on earth, about 1.2 million already discovered and named and from these only, only less than 10000 are pests to our food, fiber, health etc. which means less than 1/100. I have always been pointing to the loss of biodiversity particularly in developing countries due to unregulated use of agrochemicals [54]. One important reason behind increasing insecticides use as the most dangerous HHPs, is insecticide resistance in many insect pests [55, 56]. Resistance to insecticides by insect pests is a type of microevolution process, insects like many other life systems select for resistance to insecticides/ other types of pesticides. The most important mechanisms for insecticide resistance are "metabolic mechanisms" most by detoxification enzymes like cytochrome P-450, Glutathion s transferases etc., the level and also speciation of these enzymes increase in resistant strains as compared to susceptible ones [57,58].

HHPs and Gender issues: Because of sensitivity of the issue, we always discussed “gender and chemicals” separately. Toxic chemicals including HHPs linked to lower egg counts in women. Pregnant mothers are under threat by HHPs in developing countries. Female agricultural workers with baby on back working in paddy rice treated with HHPs in Mazandaran, Iran. Mothers milk, breast feeding is the first right for every human being and there are many reports regarding exposure of mothers to HHPs and POPs in mothers' milk [4, 49].

Pesticides More threat to children: According to my calculation during my MSc research working on pesticide residue analysis in apples, a particular amount of insecticide residue in apples that may be tolerated by an adult is harmful for a child and this depends on the body weight. This fact could be generalized for pesticide residues in all food products and particularly for vegetables and fruits. These calculations were based on Acceptable Daily Intake (ADI) and Maximum Residue Limits (MRL) determined by FAO-WHO food codex Committee. The body tolerance of a 20 Kg child is completely different than an 80 Kg adult. The other reason for lower tolerance threshold for children as compared to adults is using less diverse food by children. According to my later findings during PhD research in Canada at the university of Guelph and finding by others, consuming more diverse food and particularly more diverse vegetables and fruits inducts a higher level and more diverse metabolizing detoxifying enzymes particularly Cytochrome-P450s in the body. These metabolizing enzymes are developed more in the liver as age increases [5].

What is a HHP pesticide? According to the UNEP “Highly Hazardous Pesticides are pesticides with particularly high levels of acute or chronic hazards to health or environment according to internationally accepted classification systems such as WHO or Global Harmonized System (GHS) or their listing in relevant binding international”.

HHPs in EU countries

EU Bans Pesticides in Parks, Playgrounds, and Playing Fields; Fails to Set Organic Transition Goals in Ag (Beyondpesticides, July 2022) (6).

Banning the EU Export of Hazardous Pesticides: In Europe, many hazardous pesticides are banned from being applied to crops because of their harmful effects on humans and the environment. The European Union, however, does not prohibit the production and export of these pesticides. As a result, large volumes of these pesticides are being shipped from Europe to countries outside of the EU (7).

Written answer to the European Parliament by the EU Environment Commissioner, Mr. Sinkevičius, on the commitment to stop exports of banned chemicals:

EU: stop spreading banned chemicals! *December update: The European Commission aims to propose a law next year to stop the export of chemicals already banned in the EU, Commissioner Sinkevičius on December 1 told us himself. So now what? Now we push harder than ever before! Tones of banned chemicals are made in Europe every day and shipped all over the world. It is a massive gap in the law that makes billions for companies like Bayer-Monsanto and BASF. The European Commission committed in October 2020 to “lead by* *example, and, in line with international commitments, ensure that hazardous chemicals banned in the European Union are not produced for export." But two years on, the European Commission still has not proposed a fix. It's time for an urgent reminder: Ask EU Commissioner for Environment to get a move on and stop Europe's deadly exports. France has outlawed the export and transport of banned pesticides, so we know it can be done. Furthermore, a legal opinion by the Center for International Environmental Law says that EU exports of banned pesticides to Africa and Central America violate international treaties and human rights law. In 2018 alone, more than 81,000 tons of pesticides containing 41 different hazardous chemicals banned for agricultural use in the EU, were exported by European corporations [8].*

European Commission must not abandon promise on hazardous chemical exports ban made in EU Green Deal (November 30, 2022). Ahead of 3 December Global No Pesticides Day, more than 326 civil society organizations from across the globe, institutions and trade unions have published a Joint Statement demanding a ban on the export of hazardous chemicals that are forbidden in the EU. They urge the European Commission not to postpone the promised legislative proposal to achieve this (9).

EU regulatory issues for rodenticides: According to the ECHA GUIDANCE ON REGULATION (EU) No 528/2012 for biocides: If rodenticides (Biocide Product-type 14: Rodenticides) used outdoors in the form of baits, granulates or powder, a risk assessment for birds is necessary; EU & ECHA: European Biocidal Products Forum (EBPF). In 2002, EBPF established its Rodenticides Working Group for good practice initiatives across the rodenticides industry in Europe. Training and Certification Requirements for Rodenticides Used as Biocides in the EU, also my personal communications with the Helsinki Chemicals Forum about rodenticides regulations (10) (13).

Here in developing countries, we are facing and suffering from HHPs, every year many killed by HHP here. According to our last communications/ discussions under SAICM there are strong evidences that EU is exporting (perhaps the main exporter) HHPs to developing countries. For some years back in 20th Century for academic teaching and field crops practice we used (I myself, some others) different ecosystem services and applied ecology concepts (now under the name agroecology) for Integrated Pest Management (IPM) to control agricultural pests under Economic Injury Lines to reduce use of agrochemicals with some success [11] but now in the 21st century pesticide/ chemicals corporations increase their export of HHPs and other hazardous chemicals to developing countries according to many reports.

The Corona disaster showed that we are all in one boat on this very polluted planet now and there is no race to show up our more developed nations to developing world or worse exporting highly toxic compounds there. Having talked already in some EU countries about the problem including twice participation in the Helsinki Chemicals Forum in Helsinki [13, 14], many times I reminded ECHA and other EU agencies to give some hand to the MENA (Middle East/North Africa) countries for pesticides/ chemicals and biocides regulations. Fortunately, EU REACH is already used in many Asian countries to correct their toxic pollutions laws and regulations but not yet in the MENA countries (please see more about regulations in Asian countries in the conclusion). When in Canada about 20 years ago having some communications with the well-known environmentalist David Suzuki, according to his estimation EU countries possess the 1st rank for pesticides/ chemicals regulations and control under REACH then in the 2nd is Australia then Canada and USA at the end, where are we now in developing countries? But surprisingly just when I am at the last day reviewing this paper to send (March 29, 2023) according to our LinkedIn communication with David Law (Australian Pesticide Reduction Network), it seems that Australia is not now in a good situation for pesticide regulations and management [50].

SAICM and HHPs: I have been following SAICM (Strategic Approach for International Chemicals Management) since more than 10 years and after participation in the SAICM workshop in Cambridge UK (Sept. 2019) [12] I have been participating and discussing in nearly all SAICM Community of Practice (CoP) discussions, and will continue in 2023 under SAICM Secretariat. Our other discussions under SAICM CoP during the past 3 years have been on many other pesticides/ chemicals related issues including HHPs, pesticides/ chemicals in products, lead poisoning, chemicals and SDGs, plastics, etc., thanks to the department of environment University of Cape Town for organizing these events that are still going regularly. I have also been always following the MINAMATA convention on mercury and discussions due to the health and environmental problems of the mercury pollution. I have been following, discussing, participating and talking about any toxic pollution issues globally, regionally and nationally during the past 20 years. Other than pesticide, about any types of chemicals pollutions and regulations, PFAS, POPs and other forever chemicals, Endocrine Disruptor Chemicals (EDCs), aquatic toxicology issues, toxins, PLASTICS and microplastics, Nano toxicology, etc. and all related to loss of biodiversity in land and water in particular in wetlands and also human health issues. During Corona since 2019 we discussed these issues online nearly every day. Other than SAICM many other globally related organization, civil society, NGOs, etc., are discussing about these dangerous HHPs: FAO and WHO, IPEN, different sections of PAN, US EPA, EU ECHA are just some of them. When we look on all these, we can see that role of DEVELOPING COUNTRIES and their important related issues to HHPs are highly forgotten! For many times I proposed separate and wide discussions, actions, laws and regulations from these global agencies for pesticide regulations in developing countries [13, 14], where we are, where people suffering from HHPs and where we as related scientists do not get our parts, budgets and always are ignored. I also referred to the problem during my active participation and discussions in GMGSF of the UNEA2 in Nairobi, Kenya, 2016) (UNEP should help developing countries for pesticide/ chemicals/ biocides reduction and regulations [15, 16, 17].

Death scenarios with HHPs Globally:

More than 35 years ago I was teaching about pesticides danger and pollution in a classroom in Mazandaran Province that I heard about the BHOPAL disaster, the catastrophe affected around 500,000 people. People who were exposed are still suffering as a result of the gas leak's long-term health impacts (***The Bhopal disaster occurred on the night of December 2-3, 1984, at the Union Carbide India Ltd pesticide facility in Bhopal***). A similar case occurred in 1999 at a Peruvian town near Cuzco. Food there also was contaminated with pesticides and killed **24 children**. Bayer was found responsible for poisoning of children in Peru. Authorities say deaths and illnesses at **a primary school** in the eastern India state of Bihar are linked to an insecticide found in a midday food (18). In **2014, 122 children** lost their lives in the Muzaffarpur region, India again. So HHPs are taking lives in many developing countries including Iran while pesticide corporations are making good profits with no care about tragically situation that HHPs are doing in developing countries. Since the Bhopal disaster, later similar disasters in India, elsewhere, and remembering my own pesticide exposure during BSc and MSc research, etc. (please see below) I finally decided to put my time and efforts to help for some better and safer pesticide regulatory system for developing countries despite hardship for living expenses.

Highly Hazardous Pesticides (HHPs) History and use in Iran:

My own exposure stories: About 51 years ago when I was a third year BSc student at the department of Plant Protection (called "pest excretion" department and later and now "plant medicine!") I started my real career with good science but also with high dangerous exposure to pesticides in the gardens and crop fields. It was at first working as a summer student for related professors mostly for research projects all connected and supported by big pesticide corporation's offices in Tehran. I was in a real high exposure, at least 5 times getting close to dying mostly due to Diazinon exposure but also exposure to other Ops: not being able to focus my eyes at nights after each spraying, etc. This was 10 years after Rachel Carson wrote the book: Silent Spring 1962 [19], still no awareness in any developing countries about danger of pesticides. My exposure to Ops in Karaj apple and quince orchards: B.Sc. [20]. During my MSc research, a long 4 years project I did get the highest exposure/ doses! From Organophosphorus Insecticides: M.Sc. [21], for three years weekly spraying Diazinon and Methidathion (Supracid) in spring and summer on different apple varieties under a statistical program then sampling at the end of summer and also in Fall, many 1-2 Kg samples were transferring to the lab in deep freeze. Staying four years by a Varian 2700 Gas Chromatograph for GC analysis to find diazinon residues in different varieties of apples. My pesticide exposure continued after 1981 working on pesticide research/ practices, during teaching years.

HISTORY OF HCH USE IN IRAN, IMPACT ON HEALTH, SAFETY AND ENVIRONMENT

(History of Pesticide Use in Iran: Impact on Health, Safety and Environment) [48, 22] There is a long, strong and dangerous history of HCH/ POPs use in Iran: The horrible story of POPs polluted soil in Northeast of Iran close to Turkmenistan: About 50 years ago for few years in summer times for about 2-3 months I was there under exposure to all types of POPs sprayed by big spraying aircrafts mostly from Bulgaria and mainly on cotton fields. My main role was sampling for analysis, etc. and so had to be present in fields. Iran that time as the 4th exporter of cotton (in Iran called: white gold) globally, owned a cotton industry with most fields around two cities Gorgan and Gonbade Kavous that time partly of the Mazandaran Province by the Caspian and now in a separate Province called Golestan. Ships and ships of all types of dangerous HHPs, POPs were arriving in the Persian Gulf (mostly as technical materials) + full loaded huge trucks from EU countries (mostly as ready to use formulated and packed) and the important destinations were cotton fields, other crop fields of Gorgan, Gonbad (Mazandaran Province that time) and cotton and other field crops in the Province of Khuzistan in Southwest of Iran. Nearly all types of Chlorinated pesticides mixed with dangerous Ops like Parathion, the mixture was e.g., Turbidan (DDT + Toxaphene + Parathion!), DDT itself, Aldrin, Dieldrin (H.E.O.D), Endrin, Heptachlor, big containers were spread everywhere; this was a big business for the world pesticide corporations that time. What left behind of that big sprayings are POPs polluted soils in Northeast of Iran near Turkmenistan, this area is also called "Cancer belt" [51, 52] and in Khuzistan Province in Southwest of Iran where the sugarcane industry now is situated, only to add that now in Khuzistan where Iran main petro industries are situated, we are facing with lots of oil polluted soils as well. I mentioned the problem more than once at the site of the World Soil Day events [48].

Lindane (gamma-Hexachlorocyclohexane) - Use and history in Iran

Different formulations of Lindane are freely available for different uses in Iran now and there is a big history about Lindane in Iran, still in use in Iran legally and illegally. Like some other dangerous insecticides once awhile there are some notifications about banning of this dangerous compound but following no enforcement. Most uses of Lindane in Iran are for health but as I see there are also wide uses in agriculture against insect pests in soil. It is freely sold as head shampoo, cream, lotion, against lice. According to one of Iranian news agencies some years ago: While criticizing the Ministry of Health for using the dangerous Lindane poison in the preparation of anti-lice shampoos, the vice president of pest control and combating of the Iran Plant Protection Organization expressed hope that the dangerous poison will be removed from the cycle of human consumption as soon as possible and less dangerous poisons will be replaced. Although the Plant Protection Organization has declared the use of the dangerous Lindane poison as illegal, the Ministry of Health and Medicine has approved the use of this poison in shampoos related to eliminating head lice. And further, there are many notifications but no follow up and enforcement. Now there are many online pesticide stores that are freely selling every product that make them more profits regardless of any regulations [48].

Considering the very bad enforcement of laws and regulations in particular in agricultural sector in most developing countries we should be very worried about what is happening to our soils in developing countries. Lots of HCH, POPs [48], Highly Hazardous Pesticides, forever chemicals, add to it oil polluted soils in oil producing countries around the Persian Gulf, etc., all pressure on our soils then what will be left behind for our next generations. We need more actions from related UN agencies particularly from FAO. HHPs still in use in many developing countries are destroying whole soil biodiversity. Although we already learned a lot from EPA and later in recent years from ECHA, etc., but wondering to see that the most important World Environmental Agency allows exporting HHPs from developed world to developing countries. Here in developing countries, we are facing and suffering from HHPs, every year many killed by HHPs. According to our last communications under SAICM there are strong evidences that EU is exporting (perhaps the main exporter) HHPs to developing countries: My comment [23].

Death scenarios and environmental pollution with HHPs Ops in Iran

Highly toxic POPs plus HHPs in particular Ops like parathion, etc. in cotton fields mentioned above, other agricultural fields and citrus orchards of Mazandaran and Golestan Provinces:

Parathion Mass killing of Mazandaran: Parathion and some other Ops were widely in use against insect pests in orchards particularly citrus orchards in Iran for many years in the past and resulted in some mass killing of orchards owners particularly in the Mazandaran Province by the Caspian Sea. These incidents are partly related to the way that ordinary people look at dangerous compounds like pesticides. During my many years of teaching at the University of Mazandaran, research work and some recent talks about danger of pesticides I wondered the way that they look at these compounds, they do not want to think about how dangerous these compounds are and so handle it easily with not enough care (how pesticides are handled in developing countries, my message more than 20 years ago).

Diazinon: diazinon seems to be an inseparable OP compound from Iran pest control. Other than my own long experimental use, lab works and long GC analysis, exposure, intoxication (mentioned above), there are always lots of news about this insecticide in Iran, its wide use in crop/ orchard/ home pest control, about its ban and coming back to the market again, etc. For many years it was formulated as granule for controlling the rice stem borer *Chilo suppressalis* in rice paddies of Northern Provinces of Iran by the Caspian Sea, most of these rice paddies connected directly and indirectly to wetlands (Mazandaran is the province where the beautiful city of Ramsar is situated, where the RAMSAR Convention on wetlands started more than 50 years ago (1972). It was just a few months ago (2022) that the Plant Protection Organization of Iran announced starting ban of diazinon, should we believe it? In recent years I had some talks for Society of Wetlands Scientists (SWS) [24], for RAMSAR Convention: RAMSAR COP 13 including my side event, 2018 [25, 26], and in Changchun China, 2018 [27], etc. about loss of biodiversity in wetlands due to toxic pollution coming from connections with agricultural lands.

Toxic Threat in Asian Wetlands, a Message for the Wetlands Day 2015, my talk in the University of Kyoto: Wetlands in Asian countries are under big toxic pressure. In these countries many wetlands are connected to paddy rice fields which are the most polluted agricultural fields with different types of pesticides, other chemicals.

Entering of pesticides and chemical fertilizers in natural ponds and wetlands created a disastrous eutrophication load in most of Asian countries changing the wetlands to lagoons. The legacy situation of Anzali and recently Myankaleh wetlands in Northern Iran by the Caspian are evidences for all these problems [28].

Toxic Pollution in Wetlands and Remediation Actions, my webinar for the SWS, Oct. 2019: Wetlands are valuable ecosystems with many functions, including habitat for fish and wildlife, protecting water quality, erosion prevention, flood storage, and recreation. Wetlands are also critical habitat for many species of migratory birds, including game species that are of great ecological and economic value. Wetland fauna are threatened by toxic pollution from point and non-point sources of wastewater, that is discharged into wetlands by industry, municipalities, and other land uses within their drainage basins. Pesticides and biocides are key contaminants. Other pollutants may include, lead and metal pollution, arsenic, mercury, pharmaceuticals, toxic algae/ cyanotoxins, and generic sediment contamination. Recently, more attention has been given to toxic threats in Asian Wetlands. My message for World Wetlands Day 2015 (above) indicated that many wetlands in Asian countries are connected to rice paddies that have been polluted by various types of pesticides and fertilizers. Although direct application of Persistent Organic Pollutants (POPs) and their metabolites have mostly ceased, many chemicals persist and continue to contaminate all habitats and niches. Currently, threats from modern, toxic agents like Neonicotinoids, have resulted in diminished cohort development in birds. Remediation of toxic pollution in wetlands may be accomplished by phytoremediation, also known as phytoextraction, phytoaccumulation, or phytosequestration. Phytoremediation uses plants or algae to remove contaminants from soil or water into harvestable plant biomass and is important in constructed wetlands. Constructed wetlands recreate the functions of natural wetland plant communities, consisting of a highly diverse mix of grasses, sedges, forbs (broadleaf plants), ferns, shrubs, and trees and, at the same time, act as a filtering agent to remediate toxic pollution [29].

My course in SWS: Society of Wetland Scientists, Professional Certification Program, Professional Short Courses, COURSE INFO PROVIDER: SWSPCP Webinar, COURSE TITLE: Toxic Pollution in Wetlands and Remediation, Actions, INSTRUCTORS Ahmad Mahdavi, PhD (30).

According to my research and findings under a national project: ***Effects of highly used pesticides on Caspian Sea living organisms and natural enemies of pests*** (1995-2003) some fish species and also Gammarids (amphipods) in the Caspian Sea were affected by high usage of pesticides in the Province of Mazandaran by the Caspian Sea and also pollution that comes from Russia by Volga River (please see more in my paper in the 13th IHPA book).

Pesticide mafia in Iran like some other developing countries: Pesticide mafia, smuggling, etc. are unfortunate real facts always going in Iran as well as in some other developing countries. There are about 15 neighboring countries for Iran, a big megadiverse country, impossible to control all parts and boundaries of the country. For some years during my BSc and before MSc studies just following science particularly during my 6 months' work for a pesticide company, most of my work developing a high-tech herbicide spraying tech, I was seeing these mafia people (more than 45 years ago). Now these people own and live in multi hectare villas in US, EU and elsewhere, clearly committed crimes against people, environment and wildlife of Iran. Observing the situation of pesticide companies in Iran as a developing country during 6 months, then I did not accept work offered by two big global pesticide corporations to be their representative in Tehran, told them no and simply started my MSc studies again at the University of Tehran Feb. 1976 to follow the science, analysis about pesticides instead of making profits out of these dangerous compounds.

HHPs and worker protection in developing countries:

According to my personal information and observation for developing countries and Iran in particular, situation of both technical people (technicians) in related pesticide laboratories and pesticide formulation factories and spraying field and garden workers is not good at all, to make it more clear please see my message more than 25 years ago: How pesticides are handled in developing countries (please see the message in my paper in the 13th International HCH & Pesticides Forum Book, Zaragoza [22] or by Google search). I think the message is clear and now more than 25 years after that message, I still cannot see the situation improved. During these long years of pesticide science work, I observed acute intoxication and later death both for a laboratory technician and formulation workers. That is why now it is more than 10 years that I am calling, writing, etc. about lack of a proper: Pesticide Licensing and Certification in developing countries: If we really manage to establish and properly follow and enforce a real pesticide Licensing and Certification in any developing countries, that will improve the situation to a high extent. I have all the criteria, etc. ready, many times during our global discussions in recent years I referred to it but have not seen a real action from FAO or WHO, two agencies most responsible for these issues but are not doing enough to correct the situation [31]. Please see the discussion and conclusion section at the end of this paper about what I mean FAO, WHO and other UN related agencies need to do.

Globally wide committing suicides with HHPs with reference to the situation in Iran:

In Iran now there are lots of information online, booklets, etc. in Persian for how to avoid pesticides, cure pesticide poisonings etc. but as per my decades of experience in the field and lab, in Iran like many other developing countries there are many people killed by pesticides due to different reasons despite our decade's long activities. Biggest obstacle here is lack of cooperation between different sectors and people. I, myself a victim of pesticide poisonings, encountered some already resulted in death due to acute and also chronic pesticide poisonings (not suicidal). A porter of pesticide packages about 50 years ago in the city of Gorgan, an excellent lab technician that working in the pesticide lab of the Plant Protection Organization more than 40 years ago, younger than myself and he died about 15 years ago, and a pesticide formulation factory worker, I heard about his death later. However, there are every year many reported acute pesticide poisonings in Iran that I am aware about it, rice tablet (mostly for suicidal but also many accidental poisoning), paraquat, many Ops poisonings, etc.

Aluminum phosphide (ALP) (rice tablet) in Iran: Aluminum phosphide (rice tablet) is one the most toxic HHPs killing many people in Iran in particular in rice producing Provinces of Iran Mazandaran and Guilan, there are many references that I am including one here [32]. ALP is a lethal poison without antidote and causes cardiocirculatory collapse and has negative inotropic cardiac effect. Toxicity by ALP is caused by the liberation of phosphine gas, which rapidly causes cell hypoxia due to inhibition of oxidative phosphorylation, leading to circulatory failure (IRANTOX) [33]. Rice tablets are commonly used in Iran due to their high efficacy against rodents and insects, low cost, and availability. ALP poisoning is a common toxicity in Iran causing high mortality. This is a serious problem in agricultural region where ALP is readily available. Withdrawal of ALP tablet from the market and introduction of safer products as rodenticides and insecticides is recommended. Rice tablet (ALP) poisoning is one of the major causes of suicidal deaths in Iran and nearby countries like India, etc.

Barriers to collecting information on acute pesticide poisoning? (from our immediate SAICM CoP discussion, March 2023): Overcoming the challenges with reporting pesticide poisonings: Lack of financial resources, Lack of political will, Lack of awareness of the problem, Lack of technical expertise, Poor coordination between institutions, No surveillance or reporting systems in place, People who are poisoned do not report incidents to health or other government services, Poor or miss-diagnosis of acute pesticide poisonings, and finally poisoned individuals seek assistance from traditional healers. My discussions: For reporting chronic exposure to pesticides causing serious health problems in Iran there are good enough expertise and related information available. Fortunately, we have many reports every year by our physicians, pharmacists' toxicologists' colleagues in our society IRANTOX and some by related agricultural scientists, many scientific papers every year, many about acute poisonings. These scientific papers are about acute pesticides poisoning, some local direct/ indirect reporting by people, related offices, but unfortunately there is not one unique system to receive, control and report acute pesticide poisonings and lack of cooperation between different organizations involved and most important lack of communications of these scientific and social activities to the end pesticide users. Many digests of our discussions can be found by Google search referring to SAICM Cop, HHP, etc.

For drafting a proposal for a Severely Hazardous Pesticide Formulation for the Rotterdam Convention: In Iran we need better cooperation between many organizations now involved in pesticides, from decision making to importing and formulations, Universities/ academia, ministries of health, commerce, agriculture, related organizations, etc. Information needed for reporting HHPs to the Rotterdam Convention are: Description of the pesticide formulation, Description of the way the formulation used in the country, Clear description of the incidents related to the problem including the adverse effects [34].

Effects of HHPs on wildlife, honey bees and pollinators:

HHPs are known to be prominent drivers of pollinator decline with long term risk for pollinators. Now bits and pieces of pesticides/ chemicals and their metabolites are found in all niches and habitats preventing the natural processes of speciation responsible for the development and diversification of animals since 100s millions of years ago. A pesticide is designed to kill, when pesticide molecules and later their metabolites enter agroecosystems, they are against whole biodiversity [3]. Biodiversity is under threat in Asian wetlands due to different types of toxic pollutions like connections to agricultural lands with pesticide use, lead pollution due to millions lead bullets, mercury, and many more. Overuse and misuse of agrochemicals particularly HHPs and POPs during the past 100 years destroyed agricultural biodiversity in many developing countries due to lack regulations and enforcement. Handling toxic compounds like pesticides in developing countries have been under lots of discussions due to lack of proper methods, etc. But more important are huge differences for toxic pollution reductions and regulations between Northern and Southern countries, industries in Developed countries but more sufferings in developing countries.

Neonicotinoids (Neonics) and Pollinators: There has been shocking evidences about toxicity of Neonics for honey bees and other plant pollinators in recent years. Removal of bee-toxic pesticides and the transition to organic policies and practices are highly recommended. There are also reports about exposure and risk of fungicides on bees and a draft guidance update for public consultation on "Bees and pesticides" (PAN, Beyondpesticides, EFSA) [35]. In Minnesota USA cities seek power to ban certain pesticides due to pollinator collapse and loss of Bees, butterfly's natural habitats [36]. A study on National Pollinator Declines Blames Pesticides, Pests, and Extreme Weather [37].

Preventing wildlife poisoning

Wildlife poisoning is a big problem everywhere and most used poisons are usually HHPs. Roots of Human wildlife conflicts goes back to increasingly occupation of wildlife habitats by increasing human population, industrialism, poaching, and low income in rural areas. In Iran these conflicts already resulted in extinction of some very important species like Persian lion and Hyrcanian tiger. The Persian tiger is an endangered species whose population is less than 4,000 in natural habitats, and the Hyrcanian tiger (the Mazandaran tiger) became extinct nearly half a century ago [3].

Migratory birds under toxic threat in wetlands: In wetlands because of direct/ indirect water connections to pesticide (mostly insecticides) polluted agricultural fields there are many risky hotspots for bird poisoning by pesticides (mentioned above). Teaching for about 20 years in the University of Mazandaran, just near Ramsar city itself and observing high usage of different types of pesticide, mostly insecticides: diazinon, etc. in rice paddy fields/ other agricultural fields I call Mazandaran, a very important/ threatened breeding, wintering and stop over sites for many migratory bird species, a highly toxic polluted Province of our country Iran. I lived more than 10 years just in a city beside Freydunkenar wetland that now it is called the slaughterhouse of migratory birds. Migratory birds in Asia are under big threat of direct and indirect poisonings. Agro-ecosystems of rice paddies as well as other field crops have been under high pressure of pesticide use all over Asian countries during the past decades, with no regulations and enforcement. Heavy pesticide uses not only ended to some local disasters but also had big impact on biodiversity in Asian wetlands and so on migratory birds [59, 60].

Carbofuran: In the past there was a high usage of the dangerous insecticide Furadan based on Carbofuran, in agriculture of Iran resulting in big bird killing. According to observations in Guelph, Ont., Canada, birds falling from the sky after a long flight takes place because of entering sequestered pesticide in the blood stream of the birds (38). Also, some Ops (organophosphorus insecticides) like Organophosphate Monochrotophos, a powerful poison deadly to birds and also to people, used widely in Iran (and may be still in use) responsible for high bird's killing. While incidents of poisoning are generally declining, the mass poisoning of birds of prey reminded us all that the illegal killing of birds using banned poisons is very much a 21st Century problem. HHPs like the banned agricultural pesticide Carbofuran are extremely dangerous in even tiny quantities. Just a few grains will kill a bird of prey by breaking down its nervous system, and the poison can be absorbed through our own skin as well. A single granule of Carbofuran is capable of killing a large bird of prey and just several granules can be fatal to a human being if they were to come into contact with the banned substance.

Documentation of insecticide usages by crop and region in Iran: there is no such system in IRAN yet, how it is possible to do this when 50% of pesticide market and use is controlled by smugglers? Iran is a big and very diverse in land and culture and perhaps this diversity is an important factor preventing enforcement.

Wildlife poisoning by rodenticides in Iran – Impact of second-generation anticoagulant rodenticides on non-target wildlife: According to well documented decades long scientific research: there are disastrous impacts, Anticoagulant rodenticide (AR) poisoning has emerged as a significant concern for conservation and management of non-target wildlife. Exposure to rodenticides pose big danger for predators/ scavengers when they prey on dead/ moribund rodents. Unless under the supervision of a pest control operator / other competent person, anticoagulant rodenticides should not be used as permanent baits [39, 3].

Second generation anticoagulant rodenticides (SGARs)- SGARs are used widely in open agricultural fields in Iran. No program baiting, but permanent baiting with no later removal, and no best practice guidelines followed. Wide use in cities as biocides/ some program baiting/ awareness followed only in cities. According to the link below (in Persian [40]) from the official site of the Plant Protection Organization of Iran, up to now formulation, registration, selling and usage of nearly all types of 2nd generation anticoagulant rodenticides have been continued in Iran and there is absolutely no talk/ evidence of banning or restriction of these types of rodenticides. The only evidence that I could find is related to expression of one of minor authorities in a small city about danger of wide usage of rodenticides and nothing about ban [40, 3]. About banning of the 2nd generation anticoagulant rodenticides and also program baiting and later removing I did investigate from both Plant Protection Organization (PPO) [41] of Iran, the Iranian Research Institute of Plant Protection (IRIPP) [42] and also according to my personal information for decades on these issues, I found that in some parts of Iran these rodenticides are used only when necessary (kind of program baiting) and in some parts they are used as a preventive (no program baiting) but they are not removed later and just left in farm/ nature. About toxicity of anticoagulant rodenticide exposure and toxicosis in bald eagles (*Haliaeetus leucocephalus*) and golden eagles (*Aquila chrysaetos*), I talked some years ago in Toledo Spain for the UNEP-CMS preventing poisoning conference [3], the problem is worse in Iran, much wildlife killed by these compounds [43]. SGARs should be used only under program baiting (in which rodenticides are applied only when infestations are present, followed by bait removal) but in Iran no later removal is practiced until a few years ago. This is the reason of many bird (as well as non- target mammals) killings, a few years ago 12 golden eagles killed by poison baits in one Province of Iran (Yasooj) and many other similar cases now being reported and most online (in Persian), but just reporting and no action to prevent. Only DOE of Iran started some action and imposed high monetary fines recently [3].

In Iranian cities: Now in most Iranian big cities there are lots of programming going for rodent control because of high infestation of cities like Tehran by big rodents like *Rattus rattus* and *Rattus norvegicus.* In some cases, that I observed poison baits, most with using second generation anticoagulant rodenticides like brodifaucum, bromadiolon etc., are being removed after a period but in rural agricultural areas there is no removal, in animal husbandry farms this practice may be done that I am not aware. There are no environmental or best practice guidelines (including treatment and timing of rodent management, mitigation techniques, monitoring and evaluation, information sharing) in Iran. Related government officials are still in the primitive stage of talking only about general side effects/ environmental impacts of pesticides, but I have to add again that there are enough academic publications (most in English and in good journals like Springer, etc.) but this information rarely is read by government officials. After the revolution most of these officials came to offices only because of their religious showing, relationships to war, etc. and so rarely those good publications by academics of Iran go in action by those government officials and policy makers. As I explained above there are some recommendations in extension journals but nothing yet about guidelines/ best practices for rodenticides [3].

Poison baits used widely in Iran against wildlife

There are lots of intoxications of wildlife by poison baits deliberately by herd owners, animal husbandry farms, and illegal hunters and by feeding of wildlife on these poison baits in Iran. Installing signs/ banners etc. in places of toxic bait use, still does not exist in rural agricultural areas and it is in its starting phase in Iran. Using zinc phosphide, a traditional method to control rodents still being practiced in Iran, its usage has a long history here. Poaching is a usual behavior that had been existing in Iran, considering no laws and regulations until recently but now the government is trying to do something about it [3]. In Iran there are no poison centers for animal toxicology incidents reporting and only recently a center is starting in the medical universities of some Provinces, just starting to get some lab instruments etc. [3]

These animal poisoning centers are mainly for cases about animal feeding on poisonous plants, also for some elementary research mostly for graduate students starting their theses. There are lots of laws, regulations, and rules and fines now being announced in this regard by the DoE of Iran, there are many instances reported in newspapers and there are many reported online, but as I mentioned in other parts it seems that enforcement of these laws and regulations is very difficult in Iran as a diverse developing country. Provincial offices of the DoE of Iran all over the country are the place to report, there are now lots of incidents/ news and reports. For controlling predators by farmers there are many incidents of toxic poisoning to kill Iranian leopards, bears, wild pigs, wolves, etc. and the government, most DoE are in their early stage of trying to implement some laws and regulations and surely it takes a while to prevent intoxication of wildlife by farmers [3]. Now there is a lot of news in newspapers/ online related to many different organizations about killed wildlife like Iranian leopard, bears, birds by poison baits but no national strategies against poison baits yet. Unfortunately, in Iran /ME Countries, one is still searching and discussing on how develop guidelines and how to use materials like poison baits to be used for controlling rodents etc. in agricultural areas to produce more food for ever increasing populations in this region. Now Iran DoE is trying to do some infringement penalties for different wildlife crimes including against using poison baits. Considering that in the diverse land and environment of Iran still with high diversity of wildlife there are many offences going like killing/ trapping Iranian leopards, wolves, even very conserved cheetah now, recently many high fines are put for any usage of poison baits against wildlife. Big monetary infringement penalties being imposed for violators but still I am suspicious if there is existing enough enforcement, such as imprisonment etc. The bitter truths about the situation of biodiversity in Iran was expressed a few years ago by a very experienced environmentalist from the beautiful city of Esfahan: he expressed that during recent years more than 90-95% of Iranian biodiversity has been lost/ extinct. Then imagine what enormous negative consequences this will have for a developing megadiverse country like Iran. Biodiversity in agricultural lands in Iran and most developing countries is under big threat due to unregulated use of agrochemicals [44].

Discussions, conclusions and recommendations

Generally speaking, the situation of "chemicals regulations" in Iran, deals with politics instead of science. There are good scientific papers from academia as usual, but regulations are all under control of foreign affairs. Unfortunately, related UN conventions like BRS secretariat (Stockholm, Rotterdam and Basel), etc. love to accept, communicate, support, etc. only politically related representatives that are usually chosen by government and not people from academia and research institutions. About pesticide regulations and enforcement in Iran as I discussed many times in the past in particular in our recent past 3 years of online SAICM discussions: it is under control mostly by Ministries of Health and Agriculture. For pesticides used in agriculture, horticulture and related sections two organizations are responsible: **Institute of Plant Pests and Diseases Research** [41] mostly for research and analysis and **Plant Protection Organization** [40] for pesticide distributions, use and enforcement. There are more than 100 pesticide formulator companies, few semi-governmental. Please see also some recommendations at the end [31].

HHPs and situation of pesticides/ chemicals regulations in China and other Asian countries: During recent years in communications with REACH24 [45] and other Chinese related chemicals regulatory bodies as well as for SWS related conferences I had some travels, participations and talks in their related conferences including in the CRAC 2018 [46] in Chengdu city. CRAC 2018 that was organized with the help of EU related agencies later continued as CRACs 2019, in cooperation with Helsinki Chemicals Forum, etc. As an Iranian I am well aware of China's eager for higher and higher productions in all industries. However, it is surprising how fast China is going ahead to improve and follow generally speaking "toxic pollution regulations". The terminology that I invented and am using for developing countries: "Pesticides/ chemicals and biocides reductions and regulations for developing countries with concentration on the MENA!". Hopefully that China as well as India as two big pesticide producing Asian countries think also about more regulations in pesticide importing Asian countries including Iran. Japan is going very well as usual in toxic regulatory issues and totally speaking for all other sustainability issues in particular for related disaster reductions issues globally to save the planet. I found about this during my stay and talk about wetlands toxic pollution in Kyoto organized by UNESCO, 2015. Korea and some other Southeastern Asian countries are also going well following REACH like regulations [47] Mahdavi, Singapore 2015). Following all these regulatory issues in particular during every day online webinars, seminars, conferences plus many UNEP related conferences like UNEAS [15,16,17], Stockholm +50, etc., in person talks in many EU, Asian, African countries during the past 15 years, now I am able to help globally for these regulatory issues just to help people, environment and wildlife and free of charge. Here referring to my talk about "BIOCIDES" in Singapore 2015 with help of the ChemWatch (Chemical Watch company) I should point out the importance of "biocides regulations", now all global toxic regulatory agencies especially ECHA are going fast for biocides regulatory issues particularly overlap boundaries with pesticides that I referred to it in Singapore [47], thanks to Corona! for giving speed to the science on biocides and their regulations.

Impediments of pesticide regulations and enforcement in developing countries and how to manage it, case study Iran

What are real problems: as mentioned above the main problem is lack of a pesticide Licensing and Certification and worse is the lack of enforcement due to lack of proper infrastructures! So, the problems are big. What are existing problems and who is responsible: Main problems are discussed in my another message (from Google) more than 10 years ago: ***More exposure to a wide variety of pesticides/ chemicals in developing countries compared to developed World***: Due to the diverse numbers of pesticides/ chemicals which are imported/ produced in developing countries and used without regulations and enforcement, now people/ environment (and wildlife) in these countries are more exposed to these chemicals as compared to developed World. Many factors helped for this higher exposure, among them less knowledge and information about these dangerous compounds (please see more about this message by Google search and in my paper in the 13th Int HCH and Pesticides Forum book) [22].

HHPs High risk substances have been removed from the Iranian market!? Insecticides? Acaricides & Nematicides? Nematicides impact on wildlife?

Removed/ suspended insecticides and acaricides from the Iranian market: what about nematicides?

Diazinon- Pirimiphosmethyl- Teflobenzuron- Fuzalon- Thiaclopride- Thiomton- Fenthoat- Fention- Dicofol. Lindane- Monochrotophos- Phosphamidon- Endosulfan- Azinphosmethyl and Azinphosethyl- Carbaryl- Amitraz- Aldicarb- Emthoat- Propetamphos- Triazophos- Decrotophos- Chlorphenvinphos- Metidathion- Memtidathion- Foxim and Propoxur- Bendiocarb- Allethrin and bioallethrin- Bioresmethrin- Ciflothrin and beta-ciflotrin- Fenothrin- Lambda-cyhalothrin- Tetramethrin- Deethyl-toloamid.

Are they really removed!? Many still in market/ used, no real control/ enforcement in Iran/ other developing countries; MENA? All are unanswered questions. A few years ago (2016) vice Minister of agriculture again promised to ban dangerous pesticides and added following his talk with German authorities Iran will try to buy pesticides from German companies instead of Chinese companies.

In Iran there are many guidelines/ recommendations published in different journals related to two above mentioned organizations and also many other extension journals related to the Ministry of Agriculture, but the problem is lack of implementing and enforcing these rules and recommendations. However, there are only a few about pesticide (general information in Persian), shamefully not really enough and nothing about newly developed topics like environmental effects of rodenticides unfortunately.

What needs to be done in Iran: (some of my discussions under recent SAICM CoP): As I always mentioned we have problems of ENFORCEMENT in developing countries. A group consisting of related government agencies (here in Iran Plant Protection organization, Ministry of Health) watched by responsible people from Academics and science and all under global responsible agencies WHO, FAO, etc. and must be made mandatory in legislation (SAICM CoP, Discussion date: 30 November 2022). We need close cooperation between national authorities/ institutions and FAO, WHO, etc. to Communicate the RISK of HHPs. Country wide advocacy for farmers, gardeners, orchard owners and for public including on national TVs and radios about the risks and problem of HHPs for human and wildlife health. Also, we need close relationship and cooperation between those paper writing scientists in academia and research institutes and government agencies about the risks, local classes and demonstrations specially for more sensitive groups like pesticide/ agricultural workers, pregnant mothers and children. A worldwide action against pesticide corporations that make, promote, and export the HHPs to developing countries, more seriously than before looking for safer control methods (if pesticide corporations allow!), strict control on newly emerged pesticide corporation in Asian (China, India, etc.) and South American countries (SAICM CoP, Discussion date: 18 May 2022).

UN responsibility: the problem is that UNEP, FAO, WHO, etc. and other related UN agencies just show/ talk about the problem, known world problems like this, Climate Change, Wetlands, Pesticides pollution, etc., we need much stronger UN agencies to act immediately, seems we are losing everything now in developing countries and UN agencies except UNEP in Nairobi most are situated in developed world. Some UN related agencies instead of acting more for banning of the HHPs are making controversial treaties for example between FAO and CropLife (source PAN News [60a].

Global Action Plan on HHPs [61]: Is the Global Action Plan on HHPs necessary? (Our discussions under SAICM CoP): My response: yes of course, this is the exact question that I have been following for the past 2-3 decades following observing high negative effect of HHPs in my country Iran. Acute intoxication, hospitalization that I was hearing many times from colleagues, many deaths and also in nearby countries and globally all due to HHPs and lack of strong action by global regulatory agencies FAO/ WHO/ UNEP, etc. In fact, these UN agencies were prepared to do their best, but problems were the Big Gaps, please see my discussion some years ago about "More exposure in developing countries as compared to developed world" I already had many discussions exactly about these GAPS during the past 20 years and my talk about regulatory gaps between North and South more than 10 years ago for our IRANTOX annual conference [62]. Lack of communications between South and North, corrupt government in some developing countries and many other issues prevented these UN agencies for being able to have their efficient effects to prevent/ stop HHP in developing countries. Please see the HHPs in EU above, are really related UN agencies aware about exportation of HHPs to developing countries? And then doing nothing!

What are the key issues that you think the Global Action Plan should address? Considering the very bad situation with worker/ human mortalities due to exposure to HHPs in many developing countries and considering that developed world has much less mortality with higher pesticide usage compared to developing countries we need immediate action for much stronger enforcement procedures in developing countries with real practical actions from FAO/ WHO/ UNEP. I exactly pointed to this issue and you can find it in the GMGSF report of the UNEA2 2016 in Nairobi, also in my LinkedIn page [15,16,17]. Another important issue is the situation of obsolete pesticides in these developing countries that need stronger actions by these UN agencies [63]. Another important issue is preventing pesticide corporations for production, sale, and exportations of HHPs [7,8,9], now unfortunately some are in industrialized Asian and South American countries.

Suggestions for the best approaches to implement the Global Action Plan? Background

Obviously, the Global Plan of Action is only effective in addressing HHPs globally if it is implemented. Members are invited to come up with ideas for the implementation of the full plan, as well as components of the plan. Roles of responsible stakeholders especially FAO/WHO and governments, implementation mechanism, and monitoring should also be thought about.

Surely FAO/ WHO/ UNEP should be prepared to revolutionize! the situation of enforcement action plans to prevent HHPs in developing countries and to do this as I mentioned in my previous discussion these agencies should make sure about the existence of proper "Certification and Licensing" for pesticide use in developing countries. These UN agencies are responsible to make sure that all HHPs related/ genuine stockholders in developing countries are given possibility for expressing their idea, etc. and possibilities for action, observation, etc. in all implementation plans. We need strict control on all pesticide producing, formulation, distributing, etc. companies in developing countries by related organizations that usually are under/ called Plant Protection Organizations and so under control and watch by these UN agencies. According to my personal observations in big pest outbreaks like the locust that was going a few years ago we need more control and watch for misuse/ abuse of the HHPs, lots of damage goes to environment and wildlife. In recent years there are high research going for new/ sophisticated types/ formulations of pesticide particularly insecticides that these UN agencies should support these scientific actions for development of pesticides with non-Nerve targets. Unfortunately, there are still lots of Ops insecticides basically based on war nerve gases Sarine, Soman and Tabun in use in many developing countries. Finally, there are lots of effective biopesticides developed recently that need support and should be give special attention.

Phase-out of HHPs: Active implementation of Integrated Pest Management (IPM)

Recognition that *some HHPs are easier to phase out than others; - Availability, accessibility and affordability of alternatives, and - Buy-in from all relevant stakeholders (including farmers). Information exchange between countries was identified as important for the phasing out of HHPs. For example, sharing experiences and the feasibility of phasing out by learning from countries who have successfully phased out HHPs, such as Mozambique, how to share these experiences needs to be explored. Also, information sharing between organizations, such as the work of the PAN was suggested. Where data and capacity are limited, using decisions made by countries with a similar context was encouraged. Using JMPM HHP (Joint FAO/WHO meeting on Pesticide Management (JMPM) for HHPs criteria assists with planning for the phasing out of agriculture HHPs.*

My suggestion was to identify and eliminate the less complex ones quickly, - Another suggestion was to eliminate all HHPs that are used for suicide. All seven IPM methods that were mentioned in the discussion existed for approximately 50 years. However, it is rarely followed correctly by farmers because of industry/pesticide corporations' pressure and the exportation of HHPs from high-income countries (HIC) to low-to-middle income countries (LMICs). If the defined economic lines under IPM like Economic Injury Level (EIL), etc. are followed correctly and pest populations are managed at a lower level below these lines, HHPs won't be necessary. We practiced these in Mazandaran Province of Iran during my teaching years and it worked in a small scale however, expanding its practice to the common farmers was not possible at that time but the questions are; why FAO/ WHO did not do enough for a correct scientifically safe and practical IPM in developing countries [11]. As I am aware and tried many times in recent years FAO offices in Iran only communicate with people in the Ministry of Agriculture and nothing else, in fact their office in located in buildings of the ministry. This is the ministry that after my many times recent talks (a few months ago) to the Minister of agriculture himself about HHPs in Iran there was absolutely no response!?

Alternatives for pesticides, case study: Iran

For the past few years, I have been checking the AgroPages: https://pic.agropages.com/UploadFiles/biweekly/733479856997.html

A Chinese based online agro-news mostly about new pesticide/ IPM, etc. news in a global level. I always found so many new non-toxic pest control methods in there. I am sure that there are many more sources globally from EU, North American from USA and Canada. Also, for the past 25 years I am honored to be a member of the Entomological Society of America, receiving many weekly news, generally speaking about all these sources and I am always surprised to see so many news about successful non- toxic methods for pest control. Then why still there are mainly conventional pesticides for pest control? Perhaps FAO, WHO, SAICM, BRS, etc. should answer.

Natural products as alternatives for conventional pesticides:

In 1992 two years after my PhD in Guelph, teaching in Iran I participated and talked in the 19th International Congress of Entomology in Beijing, a big global level event [57]. Being personally interested in natural/ biological pest control due to the harm I experienced from pesticides in the past, I participated in one session about the world known neem tree, *Azadirachta indica* chaired by Dr. Ramesh Saxena from India, head of the "Neem Tree foundation". After his talks when I expressed my interest in the neem tree, he told me: Ahmad the genus name of the tree: *Azadirachta* is a Persian name basically and you have to work on this important tree in Iran (*Azadirachta* is the abbreviation for "Azad diracht" which in Persian means "Free tree", you can find the name in Google as well when you search for scientific name of the neem tree. After coming back to Iran, I immediately started to work on the neem tree, I found the tree in the biggest Island of the Persian Gulf, Qeshm Island. Later found it in the beautiful Kish Island as well, then I introduced the neem tree from the Kish Island and under the great name of the Persian Gulf, to the world with a three days big musical, painting, sport, etc. ceremony and a 2 hours TV interview in JameJam Iranian TV that time (about 25 years ago) [66, 67, 68, 69, 70]. Since then, I had been working on the tree mostly for propagation in all Persian Gulf ports and Islands, already planted 1000s of this beautiful and naturally valuable species. Please note that considering my very simple financial lifestyle I did work all these years with hardship, but naturally, saving water, climbing mountains (for the past 60 years) and supporting mountain, wetlands, etc. As I mentioned at the starting of this paper, later unfortunately the project was given to other people (usually government related). During my work that years I was also in communications with many neem trees related scientific, research people in particular professor Steven Ley from Cambridge. Professor Ley sent me lots of related his *Azadirachtin* paper works mostly about the natural product: *Azadirachtin* [65]. He was trying to synthesize the molecule, a big molecule and so very difficult. Finally, professor Ley, one of the greatest chemists globally succeeded to do the synthesis some years ago, he sent me all the processes. Honestly, I as a basically entomologist/ toxicologist did not understand the whole process [65]. My basic scientific interest on the neem tree and in particular the *Azadirachtin* came from my basic science of the: **Insect- plant interactions** that I followed deeply during my PhD studies in Guelph due to the relationships between natural pyrethrins and insects since the 100s million years ago (my PhD work was based on synthetic Pyrethroids [64,1]. I was indeed trying to see why **"insects are forewarned and forearmed to select for resistance against synthetic pyrethroids"** and finally found the answer: it was because synthetic pyrethroids molecules were based on natural pyrethrins and their historic coevolutionary relationships of natural pyrethrins to insects since the Precambrian era! that is why? In fact, I had to write many papers about these important findings but immediately returning to my country IRAN after my defense I found the situation very hard and finally retiring myself (Sept. 2003) had to leave for Canada and after some years had to come back again to IRAN, hard life. Anyway, since then about 15 years ago I was invited, participated and talked in many global events and a real big thanks to John for this event: 14th HCH…that brought me back to life after Corona. During the past 3 years Corona time, everyday working online 10-12 hours I participated in many world events, discussed, expressed my views, etc. all to help the planet.

The only safe alternative that is already in Iranian market is *Azadirachtin* based compounds, not natural one but the Azadirachtin that is imported from foreign companies and like many other insecticide/ other pesticides only formulated in Iran. In Iran there have been some incentives in the form of providing some biological control agents like *Trichogramma* tiny wasps, some advice by governmental agricultural engineers/ technicians, sporadic farm/ field schools, but I cannot recall any monetary incentives being provided. Now Iran is in its early stages of giving certificate to organic food/ crops for consideration of health of people only and no care yet about what will happen to birds etc., I think that these types of certifications need a progressed system of IPM that we do not have yet. Neonicotinoids are obviously removed/ banned from the market but as I mentioned in IRAN it takes decades for these types of compounds been removed practically, so they announce that they are removed but they are still in use, I mention again that according to government officials in official newspapers and other media only 50% (maximum) of pesticide use is under Ministry control and the other %50 are introduced/ sold by smugglers and also online sale of pesticides!

Recently the government Plant Protection Organization (PPO) announced some monitoring program for pesticides will take place but nothing in action yet and if there will be later it is for sure by PPO of the Ministry of Agriculture, the department of environment just talks, no action and no enforcement, and Ministry of Health that is the responsible body for controlling Maximum Residue Level (MRL) is putting pressure to correct the situation with no success yet. For controlling rodents by non-toxic methods there is nothing for small farm/ agriculture rodents, most in Iran are *Rattus rattus* and *Rattus norvegicus*, for big rodents there are some trappings, hunting by farmers but nothing is done yet by government as alternative to toxic rodenticides.

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References

1. Mahdavi, A., K. R. Solomon, and J. J. Hubert. 1991. Effect of Solanaceous hosts on toxicity and synergism of permethrin and fenvalerate in Colorado potato beetle (Coleoptera: Chrysomelidae) larvae. Environ. Entomol. 20: 427-432.

2. Hubert, J. J. (1984), Book, Bioassay, 2nd edition. Dubuque, Iowa: Kendall-Hunt.

3. Mahdavi, A. 2017. Preventing wildlife poisoning: case study from Iran and applicable international vs national regulatory issues, Oral presentation in the: Second Meeting of the UNEP/ CMS Preventing Poisoning Working Group (PPWG2), Toledo, Spain, 19- 21 February 2017.

4. Gender Mainstreaming for Sustainable Chemistry: gender-chemicals.org/invitation-virtual-side-event.

5. Mahdavi, A., 2007, Pesticide residues more threat to children) (Ahmad Mahdavi, Insect and environmental toxicologist, University of Guelph, Guelph, Ontario, Canada, August 19th, 2007): https://beyondpesticides.org/dailynewsblog/2007/08/new-who-report-focuses-on-children%E2%80%99s-susceptibility-to-chemicals/

6. https://beyondpesticides.org/dailynewsblog/2022/07/eu-bans-pesticides-in-parks-playgrounds-and-playing-fields-fails-to-set-organic-transition-goals-in-ag/?unapproved=324967&moderation-hash=2217a95d29807fd912b68d00725f56e9#comment-324967

7. https://www.rainforest-alliance.org/resource-item/banning-the-eu-export-of-hazardous-pesticides/

8. https://actions.eko.org/a/outlaw-exports-of-banned-chemicals?eko=true

9. https://www.pan-europe.info/press-releases/2022/11/european-commission-mustn%E2%80%99t-abandon-promise-hazardous-chemical-exports-ban

10. EBPF – European Biocidal Products Forum: https://circabc.europa.eu/sd/a/cb0765b5-e7b0-4c17-9e42-8ade4421227d/CA-March17-Doc.5.3.a%20-%20RWG-Proposal%20for%20maximum%20rodenticide%20pack%20sizes%20for%20general%20public.pdf

11. Introduction to Insect Pest Management, 3rd Edition: Robert L. Metcalf (Editor), William H. Luckmann (Editor), ISBN: 978-0-471-58957-0 July 1994 672 Pages.

12. Technical Working Group on targets, indicators and milestones for SAICM and the sound management of chemicals and waste beyond 2020: University of Cambridge, Cambridge, UK, Sept. 3-5 2019. http://www.saicm.org/Beyond2020/TechnicalWorkingGroup/tabid/8287/language/en-US/Default.aspx http://www.saicm.org/Portals/12/Documents/meetings/Indicator-WS/IndicatorWorkshop_participantslist_FINAL.pdf

13. Mahdavi, A. 2017. Active participation and discussions in the: Helsinki chemicals forum 2017, ECHA (European chemicals Agency). 8-9 June. Helsinki, Finland.

14. Mahdavi, A. 2016. Active participation and discussions in the: Helsinki chemicals forum 2016, ECHA (European chemicals Agency). 25-27 May. Helsinki, Finland.

15. Mahdavi, A. 2016. Active participation in the UNEA2, the World environment assembly. May 2016, Nairobi, Kenya.

16. Mahdavi, A. 2016. Global Major Groups Stakeholder Forum (GMGSF), UNEA2, 21-22 May, Nairobi, Kenya. UNEP should help developing countries for pesticide/ chemicals/ biocides reduction and regulations: https://www.linkedin.com/pulse/unep-should-help-developing-countries-pesticide-biocides-mahdavi?trk=prof-post

17. Mahdavi, A. 2016. Science policy forum, UNEA2, 19-20 May, Nairobi, Kenya.

18. School meal tainted by pesticides kills Peru kids - NBC News: https://www.nbcnews.com/wbna44621845: and Pesticide confirmed in deadly Indian school lunch: https://www.washingtonpost.com/world/2013/07/18/

19. Rachel Carson, the book: Silent Spring, 1962: https://en.wikipedia.org/wiki/Silent_Spring

20. Mahdavi, A. 1981. MSc Thesis. Study the biology of quince moth, *Euzophora bigella* Zeller (Lepidoptera: Pyralidae) in Karaj and testing four insecticides to control it. Dept. of Plant Protection, College of Agriculture, University of Tehran (Karaj). Iran.

21. Mahdavi, A. 1974. Determination of residue of Diazinon in different varieties of apple fruit by Gas Chromatography in two methods of spraying, fixed program and surveying program. Dept. of Plant Protection, College of Agriculture and college of Health, University of Tehran. Iran.

22. Mahdavi, A. 2015. History of Pesticide Use in Iran: Impact on Health, Safety and Environment. Forum book of the: 13th HCH & Pesticides Forum Nov. 3-6, Zaragoza, Spain. http://www.ihpa.info/docs/library/forumbooks/13/13thHCHForum_FORUM_BOOK_2015.pdf at Pages 168- 173.

23. https://news.climate.columbia.edu/2013/04/01/phosphorus-essential-to-life-are-we-running-out/

#:~:text=Phosphorus%20cannot%20be%20manufactured%20or,are%20running%20out%20of%20phosphorus
24. Society of Wetlands Scientists (SWS): https://www.sws.org.
25. Mahdavi, A. 2018. Side Event preparation and presentation: Effects of agrochemicals on wetlands biodiversity in Asia: case study Mazandaran (by the Caspian Sea, Iran, (RAMSAR), Raising awareness on the situation of toxic pollution in wetlands and its effects on biodiversity. 13th Meeting of the Conference of the Parties to the Convention on Wetlands, Dubai, United Arab Emirates, 21-29 October 2018.
26. Mahdavi, A. 2018. Active participation in the: 13th Meeting of the Conference of the Parties to the Convention on Wetlands (RAMSAR). Dubai, United Arab Emirates, Oct. 21-29.
27. Mahdavi, A. 2018. Oral presentation: Negative Impact of pesticide use in rice paddies on nearby wetlands: case study Mazandaran (Iran, by the Caspian). 2018 SWS - China Chapter and Asia Chapter Joint Meeting, Wetlands and Ecological Civilization, Northeast Institute of Geography and Agroecology (IGA), Chinese Academy of Sciences, Changchun. China, August 17-21.
28. Mahdavi, A. 2015. Toxic threat to Asian wetlands: a message for the wetland's day 2015. Oral presentation in the: UNESCO International Symposium on Scientific, Technological and Policy Innovations for Improved Water Quality Monitoring in the Post-2015 SDGs Framework. July 15-18 Otsu Kolabo- Shiga and Kyoto University, Kyoto, Japan. https://www.youtube.com/watch?v=iHne4MfJRvo
29. Mahdavi, A. 2019. Toxic Pollution in Wetlands and Remediation Actions, My webinar for the SWS, Oct. 2019: https://www.youtube.com/watch?v=5FLF_sL00-g
30. Mahdavi, A. COURSE TITLE: Toxic Pollution in Wetlands and Remediation, Actions, INSTRUCTORS Ahmad Mahdavi, PhD. https://www.wetlandcert.org/shortcourse_details.html?cid=SWS00001W-068
31. Mahdavi, A. (2010) In proceedings of the conference on: Half century of the pesticide uses in Iran, 2-3 March 2010, Tehran, Iran.
32. IranTox. Iranian Society of Toxicology: http://irantox.net/English.aspx
33. Hosseinian A. et al. 2011. Aluminium phosphide poisoning known as rice tablet: a common toxicity in north Iran.
34. PROCEEDINGS of the Regional Workshop for the Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade Tehran, Iran, 19-23 October 2002.
35. https://www.efsa.europa.eu/en/news/bees-and-pesticides-draft-guidance-update-public-consultation.
36. https://www.startribune.com/minnesota-cities-seek-power-to-ban-certain-pesticides-amid-pollinator-collapse/600036974/
37. https://beyondpesticides.org/dailynewsblog/2023/02/study-on-national-pollinator-declines-blames-pesticides-pests-and-extreme-weather/
38. Book: The use and significance of pesticides in the environment. [1979]. McEwen F.L.; Stephenson G.R.
39. Mourad, et al, 2012, Anticoagulant Rodenticides on our Public and Community Lands: Spatial Distribution of Exposure and Poisoning of a Rare Forest Carnivore.
40. http://ppo.ir/LinkClick.aspx?fileticket=vjzmXSwZqvY%3d&tabid=808
41. http://ppo.ir/Default.aspx
42. http://portalold.iripp.ir/EnglishPages/Englishindex.htm
43. Niedringhaus, KD, 2021. Anticoagulant rodenticide exposure and toxicosis in bald eagles (Haliaeetus leucocephalus) and golden eagles (*Aquila chrysaetos*) in the United States.
44. Report of the first meeting of the Assessment Working Group of the International Network of Soil Pollution (INSOP)
45. REACH24H Consulting Group (China): https://www.reach24h.com/en
46. Mahdavi, A. 2018. Oral presentation: Pesticide Registration and Market Analysis in Iran and Middle Eastern Countries, Global Pesticide Registration Session, 10th Chemical Regulatory Annual Conference (CRAC 2018), REACH24H Consulting Group. Shangri-La Hotel, Chengdu, China, September 9-12.
47. Mahdavi, A. 2015. Regulation of biocides in Middle Eastern countries case study: regulation of biocides in Iran, Oral presentation in the conference: Biocide Asia 2015. Singapore, 28-29 Sept.
48. John Vijgen, et al, 2018 The legacy of pesticides and POPs stockpiles—a threat to health and the environment, Environmental Science and Pollution Research volume 25, pages31793–31798 (2018)
49. Gender and Chemicals, Dr. Minu Hemmati, gender-chemicals.org: http://gender-chemicals.org
50. David Low, our communications in LinkedIn: https://www.linkedin.com/in/david-low-00845544?miniProfileUrn=urn%3Ali%3Afs_miniProfile%3AACoAAAlSJsgBT1vr0WzZT9_IjOt4rs0VmOWUFvo&lipi=urn%3Ali%3Apage%3Ad_flagship3_detail_base%3B53%2Fq2PxGSJiWAV%2FL%2BoUz2Q%3D%3D
51. Mahdavi, A. 210. Methodology for medical geology studies in developing countries. Full paper in: proceedings of the 1st international symposium of medical geology. 14-16 June 2010, Tehran, Iran. The Report of the First International Symposium on Medical Geology Geological Survey of Iran Tehran, Iran.

52. Mahdavi. A. 2011. Medical Geology and its importance for developing countries. Paper presented in the 11th International Congress of the Iranian Society of Toxicology. Proceedings of the 11th International Congress of the Iranian Society of Toxicology, September 2011, Mashhad, Iran.
53. Mahdavi, A. 2017. Oral presentation: Loss of biodiversity in mountains. Food and Agricultural Organization of the United Nation (FAO), Mountain Partnership Global Meeting 2017, December 10-13, Rome, Italy.
54. Sharma, Anket, 2019. Worldwide pesticide usage and its impacts on ecosystem
55. Mahdavi, A. 2000. Metabolic mechanism (s) of resistance of Colorado potato beetle, *Leptinotarsa decemlineata* Say to synthetic pyrethroids. Proceedings of the conference: Insect toxicology 2000, Berkeley, California.
56. Mahdavi, A. 1995. Combined LD50s, a computerized method for calculating and comparing LD50s and Synergistic Ratios. Proceedings of the 12th Plant Medicine Congress of Iran. Karaj, Iran.
57. Mahdavi, A. 1992. Microsomal NADPH-oxidation (in the absence and presence of cytochrome c) and NADPH-cytochrome c(P-450) reductase activity in adults of the resistant and susceptible strains of Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). Proceedings of the Nineteenth International Congress of Entomology. Beijing, China.
58. Mahdavi, A. and K. R. Solomon. 1988. Synergism of permethrin and fenvalerate in freshly-molted fourth-instar larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) by piperonyl butoxide. Proceedings of the Eighteenth International Congress of Entomology. Vancouver, Canada.
59. Protection of migratory birds in Myankaleh wetland by the Caspian Sea, Mazandaran, Iran: https://www.worldmigratorybirdday.org/events/2022/protection-migratory-birds-miankaleh-wetland-caspian-sea-mazandaran-iran
60. Migratory Birds Preventing Poisoning: https://www.worldmigratorybirdday.org/events/2018/migratory-birds-preventing-poisoning
60a. PAN News December 3, 2021 - 08:59 https://www.pan-europe.info/press-releases/2021/12/un-fao-urged-abandon-controversial-pesticide-industry-partnership-more-187300
61. Global Action Plan on HHPs: https://saicmknowledge.org/sites/default/files/meterial/Draft_Global_Action_Plan_HPP.pdf
62. Mahdavi, A. 2009. Bridging the gap between South and North for pesticide/ chemical regulations and research. Proceedings of the 10th Iranian congress of toxicology, 18-20 May 2009, Tehran, Iran.
63. Mahdavi, A. 2011. Participation in the TOT, FAO Training of Trainers on Obsolete pesticides inventory and safeguarding. Nov. 2011, Izmir, Turkey.
64. Mahdavi, A. 1991. Metabolic mechanisms of resistance of the Colorado potato beetle (Coleoptera: Chrysomelidae) to synthetic pyrethroids, permethrin and fenvalerate. Dissertation: Thesis (Ph. D.) --University of Guelph, 1990. Publisher: Ottawa: National Library of Canada = Bibliothèque nationale du Canada, 1991. Series: Canadian theses = Thèses canadiennes. Edition/ Format: Thesis/dissertation: Thesis/ dissertation: Microfiche: English.
65. Steven V. Ley, et al, 2008. The Synthesis of Azadirachtin: A Potent Insect Antifeedant.
66. Mahdavi, A. 2015. Pesticidal plants for vector control, oral presentation in the workshop: Regional workshop on sound life-cycle management of DDT under the Stockholm Convention, 24-26 November 2015, Nairobi, Kenya.
67. Mahdavi, A. 2015. Comparison of Azadirachtin content in neem tree, *Azadirachta indica* Juss seeds collected from four different parts of the Persian Gulf. Poster presentation in the: 13th Iranian International Congress of Toxicology, 12-14 May, Urmia Lake Research Center, Urmia University. Urmia, Iran.
68. Mahdavi, A. 2014d. Feasibility study for cultivation and propagation of the neem tree, *Azadirachta indica* in the Mahshahr Petrozone. 4th Report (report for study, executive and experimental phases). 50pp. Feb. 2014. Mahshahr Petrozone, Mahshahr, Iran.
69. Mahdavi, A. 2012b. The Miraculous Neem tree, *Azadirachta Indica* in Western Persian Gulf. Third Danai Festival of the Persian Gulf. The Persian Gulf Celebration Week, April 29- 6 May. Bushehr. Iran.
70. Mahdavi, A. 2012. Neem in the Persian Gulf. Farming Matter, March 2012. Farming Matters is published by ILEIA, the Centre for Learning on Sustainable Agriculture. ILEIA is a member of AgriCultures, a global network of organizations that share knowledge and provide information on small-scale, sustainable agriculture worldwide. www.farmingmatters.org.

TOMATO AND OLIVE MICRONUTRIENTS AS "HUMAN BODY REMEDIATION" IN PEOPLE LIVING IN CONTAMINATED AREAS: FOCUS ON Β-HEXACHLOROCYCLOHEXANE

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Summary

β-Hexachlorocyclohexane (β-HCH) is a waste product of the synthesis of technical-grade lindane (γ-HCH), a widely used pesticide until the 1970s-80s. In comparison to the other members of the hexachlorocyclohexane family, β-HCH exhibits a high chemical stability and long half-life, leading to a substantial environmental impact. In the field of chemical carcinogenesis, we have focused our attention on the cellular mechanisms of β-HCH, identifying the biotargets that mediate its pleiotropic effects. These bio-targets are also valuable for identifying natural substances with inhibitory activity against β-HCH- toxicity. These findings could serve a starting point for including functional food into the diet of individuals living in contaminated areas, aimed at protecting their health.

Keywords

β-Hexachlorocyclohexane, STAT3, Carcinogenesis, Natural Compounds, Environmental Pollutants.

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Introduction

The term lindane is the common name for an organochlorine pesticide widely used in the 1960s-70s and it is permanently banned by Stockholm Convention on persistent organic pollutants in 2009. The chemical nomenclature of lindane is γ-hexachlorocyclohexane (γ-HCH), which designates one of the five isomers (α,β,γ,δ,ε) obtained through the benzene chlorination in the presence of UV light. The raw product from the reaction contains about 10-15% of γ-HCH (lindane), the only hexachlorocyclohexane derivative endowed with pesticidal activity, whereas the remaining proportion is constituted by inactive isomers, namely: α-HCH 60-70%, β-HCH 5-12%, δ-HCH 6-10%, ε-HCH 3-4%. As can be deduced from these data, the percentage yield of γ-HCH is very low[1]. The lindane production process has a huge impact on the environment because it leads to a considerable amount of pollution as a result of its illegal disposal[2]; the extent of the contamination is an issue of such magnitude that the Earth has been dubbed "the lindane sink" by a team of Indian researchers who estimated that there are 7 million tons of HCH isomers on our entire globe. Among all the isomers, β-HCH is the most persistent, accounting for the highest percentage currently found in lindane landfill sites. The equatorial arrangement of the six chlorine atoms in the β-HCH structure, in fact, confers it low reactivity and therefore high stability (half-life of about 30 years); these chemical-physical characteristics have earned it the definition of *"fossil isomer*". Its metabolic stability and bioaccumulation tendency explain why β-HCH is the isomer most frequently detected in the blood of people living in polluted areas. The attention of the scientific community towards the risk to human health was mainly focused on the toxicity of the pesticide lindane, the γ-HCH; however, in the late 1990s'early 2000s, the increasing awareness of β-HCH presence in the food chain put the spotlight on this very little-known isomer. Despite β-HCH has been the subject of some epidemiological studies since then, there still were major gaps in the knowledge of this compound. In 2015, our research group embarked on the challenging study of the molecular mechanisms activated by β-HCH in human cells Figure 1.

This interest arose in 2005, when β-HCH was found in cow's milk produced by local farms during a random national survey on chemical contamination carried out in the industrial area of Sacco Valley, in the south of Rome[3].

The traceable cause for this disaster was the illegal dumping of chemical wastes originating from the lindane manufacture; β-HCH, as part of unwanted by-products, was massively discarded by the industry that produced lindane, near the banks of the Sacco River, which unwillingly played the role of "β-HCH diffuser" in the valley area. In 2016, the results of an epidemiological study carried out on the population living along the river were published. The biomonitoring data were crucial for our research because they provided us with the relative blood concentration of HCH in exposed population, the average of these values was used in our experiments, allowing us to reproduce the real exposure conditions.

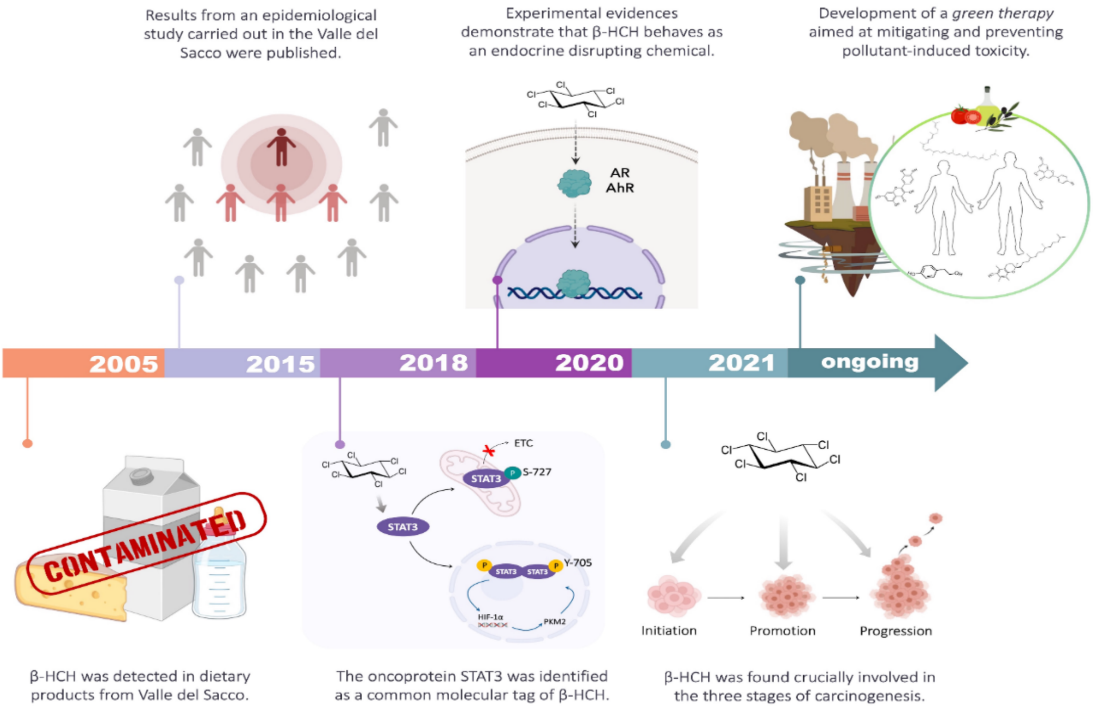

FIGURE 1. TIMELINE OF OUR STUDIES ON CELLULAR TOXIC EFFECTS INDUCED BY Β-HCH: PAST, PRESENT AND FUTURE

In the first place, the aim of our research was to elucidate the molecular-cellular mechanisms that might be the culprits of β-HCH-related diseases described in epidemiological studies, as there was data on this topic in the scientific literature. We initially identified the oncoprotein STAT3 as a molecular tag of β-HCH toxicity; STAT3, in fact, has been proven to be a hub protein at the crossroad of β-HCH-induced molecular responses in a panel of cell lines representative of prostate, breast, and liver cancer3. Going forward in the molecular study on the cellular activity of β-HCH, we also investigated whether it displayed other biochemical features typical of the organochlorine pollutants family, such the behavior as endocrine disruptors, the capability of triggering the Aryl Hydrocarbon Receptor and the promotion of oxidative stress via ROS production. The results confirmed that β-HCH exhibited all of these cellular activities4. Finally, we demonstrated that β-HCH could induce some cellular responses underlying the three phases of the carcinogenesis5. These studies have enabled us to identify the cellular biomarkers of β-HCH which, in addition to be useful as potential biotargets for molecules with inhibitory activity, can be predictors of possible non-communicable diseases that may develop in people with this dangerous inheritance. The focus of our study has then shifted on the potential employment of natural molecules to counteract β-HCH negative cellular impact. In particular, we took into account plant-derived compounds that are modulators of the validated β-HCH intracellular targets and that can easily be introduced with the diet. This strategy could be described as a "green therapy" aimed at protecting the health of contaminated populations6. This approach, also conceived as a remediation of the human body, is inexpensive, feasible, and may offer a protective shield for the organisms of exposed individuals pending land reclamation, which are expensive projects with long resolution times. The study of the scientific literature enabled us to select a wide pool of natural compounds with a well-established inhibitory activity towards STAT37, aryl hydrocarbon receptor, reactive oxygen species (ROS), and steroid receptors. In designing experimental schemes to test the inhibitory and, thus, the protective effects of these natural products against β-HCH-induced toxicity, we must consider that β-HCH has a pleiotropic activity in the cell; for this reason, a greater protective efficacy could be achieved by administering a mixture of natural substances, rather than a single compound, in order to obtain the simultaneous inhibition of multiple biotargets. From the extensive study of cross-referenced data deposited in open-access databases, we found out that suitable candidates for the development of an effective green therapy against β-HCH were bioactive compounds mainly contained in tomatoes8 and olive oil 9. We were searching for an easy-to-administer preparation, and we came across a EU patented (No. EP2851080A1 by Janus Pharma) food supplement, which ideally fits our purposes, referred to as Tomato and Olive Bioactive Compounds (TOBC)10,11. In a first step, we tested this food supplement on a cellular model that recapitulates all the biomarkers of β-HCH, the hormone-responsive prostate cancer cell line LNCaP. As demonstrated in our published manuscript, TOBC acts as a shield against all the toxic actions of β-HCH12. These results fuel the hope that with appropriate supplements and/or specific diets, pollutant-related diseases can be mitigated or prevented. To complement the cellular studies on the protective effect of TOBC against β-HCH toxicity, we thought it opportune to assay it on additional cell lines representative of tumors such as breast (MCF-7) and liver (HePG2) cancers. It is important to underline that these cellular models were the same we employed to identify the central role of STAT3-mediated pathways in response to β-HCH insult3. To test the efficacy of TOBC in these two cell lines, we chose to use the immunofluorescence, an assay that allows us to show the cellular distribution of a protein. Thus, STAT3 nuclear localization represents an indicator of its activation; indeed, the inhibition of STAT3 pathways by the active compounds constituting the TOBC, will prevent its nuclear translocation. The experimental scheme for processing MCF-7 and HepG2 cells is the same as in the previous research on LNCaP3,4,12: Obtained results are shown in Figure 2 in these images, representing both the cell lines, the nuclear presence of STAT3 is clear in the β-HCH-treated samples, whereas the protein is cytosolic in the controls and in those treated with TOBC. These outcomes confirm the protective activity of TOBC in these two cell lines as well, which is precisely demonstrated by the inhibition of STAT3-mediated pathways.

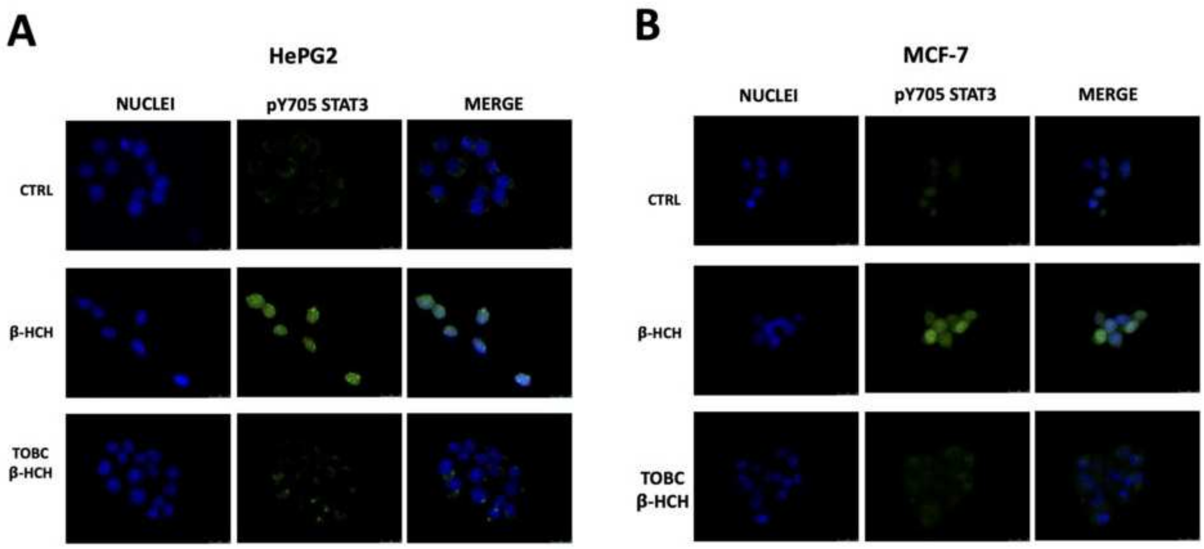

FIGURE 2. IMMUNOFLUORANCE ASSAY.
Images clearly show STAT3 nuclear localization in cells treated with only β-HCH, but not in samples co-treated with TOBC and β-HCH. Selected images reported are representative of three independent experiments and were captured under same acquisition parameters.

The activation of STAT3, induced by β-HCH has the immediate effect to promote and sustain proliferation[3], causing uncontrolled cell growth. Moreover, the proliferative phenomenon is one of those responsible for the production of ROS species, i.e. the induction of oxidative stress and consequently oxidative damage to biomolecules such as DNA. In order to verify this effect, we performed the MTT assay on cells treated with either β-HCH or TOBC and co-treated. Variations in ROS levels were determined by immunofluorescence using the CELLROX reagent and finally through comet assay we evaluate if ROS species induced genotoxic DNA damage. Obtained results on HepG2 and MCF-7 cells are shown in Figures 3, 4, 5 and confirm previously observed evidence of a protective effect of TOBC against β-HCH-induced cell toxicity. Indeed, as shown in the images of the co-treatments, there is evidence of reduced proliferative activity and a decrease in both ROS species and DNA damage.
The experiment was repeated three times with similar results, and the obtained values are presented as the mean and standard deviation (Figure 3). Statistical analysis was performed with GraphPad Prisma software using Student's t-test.

Statistically significant differences (** $p < 0.01$; *** $p<0.001$; **** $p < 0.0001$;) are marked with asterisks and refer to the untreated HePG2 and MCF-**7** cells used as the control (CTRL).

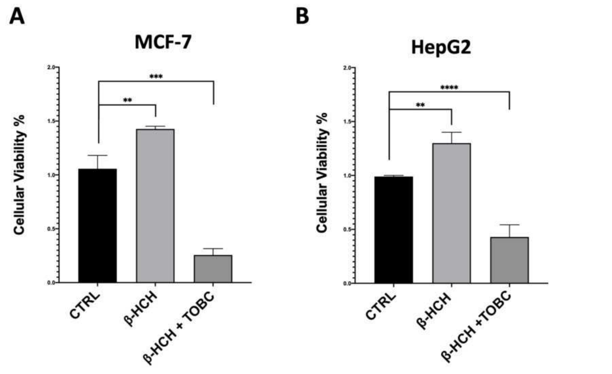

FIGURE 3: MTT ASSAY.

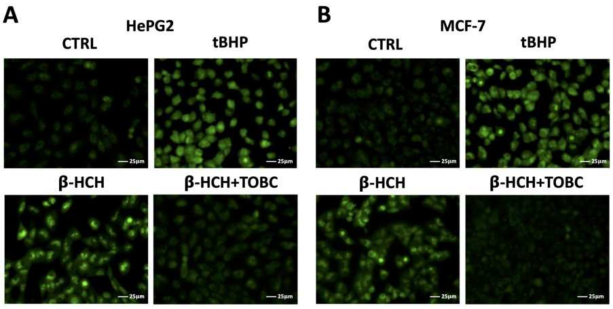

FIGURE 4: DETECTION OF ROS WITH CELLROX® REAGENT.

TOBC exhibits antioxidant activity towards reactive oxygen species (ROS), in HePG2 (**A**) and MCF-7 (**B**) cells line generated by β-HCH. Images and relative fluorescence clearly show a reduction of ROS in presence of co-treatment TOBC and β-HCH.

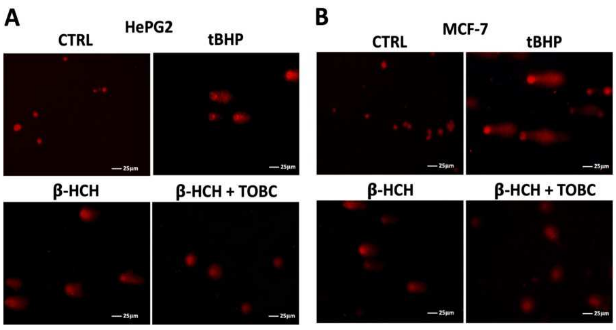

FIGURE 5: DNA DAMAGE WAS EVALUATED USING COMET ASSAY.

As shown in this figure, DNA fragmentation, in HePG2 (**A**) and MCF-7 (**B**) cells-line caused by β-HCH is reduced in presence of TOBC; 75 µM tBHP for 1h was employed as a positive control for DNA fragmentation. CTRL: untreated cells; tBHP: positive control; β-HCH.

Conclusions

Over the past eight years, our scientific efforts have been aimed at contributing to the advancement of knowledge in the field of chemical carcinogenesis[13]. Our cellular studies, in fact, focused on the comprehensive characterization of the organochlorine pesticide β-HCH to elucidate the molecular mechanisms underlying its cellular activities across non-transformed and cancerous cell lines. Throughout the timeline of our research, our first milestone was indeed the identification of a common cellular target among the different cellular and tumor types in order to acquire a reliable biotarget. This accomplishment paved the way to in-depth investigate the capability of natural or synthetic substances to inhibit the identified biotarget, thus exerting a protective action against β-HCH-induced damage. Our first characterized biotarget was the STAT3 protein, which turned out to be the central protein modulating cellular responses activated by various ß-HCH-sensitive receptor pathways[3]. Subsequently, we verified whether β-HCH triggered other processes such as: activation of Aryl Hydrocarbon Receptor and Steroid Receptors, oxidative stress, and energetic metabolic shift, much like other OCPs. In this context, we then identified additional biotargets, broadening the availability of molecular targets that we could use as a shotgun for inhibitors with protective activity against β-HCH toxicity. Lastly, to clarify β-HCH involvement in chemical carcinogenesis, we investigated the molecular mechanisms of β-HCH on the normal bronchial epithelium cells BEAS-2B[5].

The identification of a panel of cellular biotargets for an environmental pollutant is an important step; In the broader field of cellular biochemistry research, identifying a target or and/or biomolecular marker is crucial for understanding biological mechanisms, predicting disease, and identifying synthetic or natural inhibitors. In our case, biotargets for ß-HCH were explored to select a pool of natural substances for targeting them. Consulting the scientific literature, we identified numerous natural compounds that could inhibit these biotargets. Given our objective to select compounds found in vegetable products with cost-effective production, easy usage, and implementation through dietary means, we focused on those present in tomatoes and olive oil, which we found in a particular preparation[14]. The results of our cellular studies conducted on three cell lines representing different organs and tumors are highly encouraging. In fact, in all the samples treated with TOBC, a protective effect against the cellular damage caused by β-HCH is clearly evident. These results are groundbreaking in the field of chemical carcinogenesis and represent a valid support for a project intended at intervening in health policies related to prevention and treatment for population groups facing pollution-related issues. This approach is easily applicable and could potentially provide the basis for large-scale "green therapy" initiatives to protect the health of people living in polluted areas. Such initiatives can serve as preventative measures, as defenses against the onset of disease, and as adjuvants in pharmacological therapies for individuals contaminated with OCPs or other pollutants.

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References

1. Vijgen, J. *et al.* Hexachlorocyclohexane (HCH) as new Stockholm Convention POPs—a global perspective on the management of Lindane and its waste isomers. *Environmental Science and Pollution Research* 18, 152–162 (2011).
2. Vijgen, J., de Borst, B., Weber, R., Stobiecki, T. & Forter, M. HCH and lindane contaminated sites: European and global need for a permanent solution for a long-time neglected issue. *Environmental Pollution* 248, 696–705 (2019).
3. Rubini, E. *et al.* STAT3, a Hub Protein of Cellular Signaling Pathways, Is Triggered by β-Hexaclorocyclohexane. *Int J Mol Sci* 19, 2108 (2018).
4. Rubini, E. *et al.* β-Hexachlorocyclohexane: A Small Molecule with a Big Impact on Human Cellular Biochemistry. *Biomedicines* 8, 505 (2020).
5. Rubini, E. *et al.* β-Hexachlorocyclohexane Drives Carcinogenesis in the Human Normal Bronchial Epithelium Cell Line BEAS-2B. *Int J Mol Sci* 22, 5834 (2021).
6. Hoffman, J. B. & Hennig, B. Protective influence of healthful nutrition on mechanisms of environmental pollutant toxicity and disease risks. *Ann N Y Acad Sci* 1398, 99–107 (2017).
7. Yang, J., Wang, L., Guan, X. & Qin, J.-J. Inhibiting STAT3 signaling pathway by natural products for cancer prevention and therapy: In vitro and in vivo activity and mechanisms of action. *Pharmacol Res* 182, 106357 (2022).
8. Ali, M. Y. *et al.* Nutritional Composition and Bioactive Compounds in Tomatoes and Their Impact on Human Health and Disease: A Review. *Foods* 10, 45 (2020).
9. Jimenez-Lopez, C. *et al.* Bioactive Compounds and Quality of Extra Virgin Olive Oil. *Foods* 9, 1014 (2020).
10. Cormio, L. *et al.* Improvement of urinary tract symptoms and quality of life in benign prostate hyperplasia patients associated with consumption of a newly developed whole tomato-based food supplement: a phase II prospective, randomized double-blinded, placebo-controlled study. *J Transl Med* 19, 24 (2021).
11. Quiros-Roldan, E. *et al.* Symptoms and quality of life in HIV-infected patients with benign prostatic hyperplasia are improved by the consumption of a newly developed whole tomato-based food supplement. A phase II prospective, randomized double-blinded, placebo-controlled study. *J Funct Foods* 82, 104495 (2021).
12. Rubini, E. *et al.* Tomato and Olive Bioactive Compounds: A Natural Shield against the Cellular Effects Induced by β-Hexachlorocyclohexane-Activated Signaling Pathways. *Molecules* 26, 7135 (2021).
13. Kabirai, A., Alka Chahar, Chahar, N. & Gupta, J. Chemical carcinogenesis: A brief review on mechanism & metabolism. *Journal of Oral Medicine, Oral Surgery, Oral Pathology and Oral Radiology* 6, 120–124 (2020).
14. Natali, P. G., Piantelli, M., Minacori, M., Eufemi, M. & Imberti, L. Improving Whole Tomato Transformation for Prostate Health: Benign Prostate Hypertrophy as an Exploratory Model. *Int J Mol Sci* 24, 5795 (2023).

THE USE OF THERAPEUTIC AGENTS DERIVED FROM THE PLANTS AND FRUITS GROWING IN KYRGYZSTAN FOR THE ELIMINATION OF ORGANOCHLORINE PESTICIDES FROM GASTROINTESTINAL TRACT OF NURSING WOMEN

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Summary

The paper presents the results of analysis for organochlorine pesticides (OCPs) presence in breast milk, blood and urine of urban nursing women residents of Kyrgyzstan before and after the treatment with therapeutic agents derived from local medicinal plants and fruits. The first test group included 27 women who were treated with a concentrate derived from local medicinal herbs for the neutralization and elimination of OCPs from gastrointestinal tract (GT), and the second test group involved 24 mothers who didn't administered the concentrate. The breast milk, blood and urine samples were examined for the presence of OCPs: hexachlorocyclohexane (HCH) in the form of its α-, β-, γ-, and δ-isomers; 4,4'-dichlorodiphenyltrichloroethane (4,4'-DDT), 4,4'-dichlorodiphenyldichlorethylene (4,4′-DDE), 4,4′-dichlorodiphenyldichloroethane (4,4′-DDD), aldrin, dildrin, and heptachlor, eventually, eight of them were detected, excluding δ-HCH and dildrin.

A 10-12 day administration of therapeutic agents produced from local medicinal plants, including endemic herbs and fruits made it was possible to reduce the concentration rates of hexachlorocyclohexane (HCH) - α-, β-, γ isomers and dichlorodiphenylethylene (DDE) in breast milk. Concentration level of HCH decreased by 2.05 times, but the detection rates remained unchanged, DDE rates decreased from 88.9% before the start of the treatment up to 66.7%, P = 0.551 after the course of treatment, concentration level decreased by 2.01 times. The level of HCH in blood samples decreased from 33.3% to 22.2%, P = 0.29, concentration decreased by 53.5 times. DDE levels remained at 55.5% even after the course of treatment, but its concentration decreased by 28.5 times.

The level of HCH in urine samples increased from 11.1% before the treatment to 22.2% after the course of treatment, but concentration level decreased by 58 times, DDE - from 33.3% before the treatment to 3.7% after the treatment, and only DDE traces were detected, P=0.0004. In the second group (control group), the levels HCH and DDE in breast milk samples and HCH level in blood samples remained at 66.7%, in urine samples - remained unchanged. DDE level in breast milk, on the contrary, increased from 50.0% to 66.7%, in urine - the level of HCH remained at 50%, and DDE - increased from 12.5% to 16.7%.

Thus, the use of therapeutic agents obtained from medicinal plants, including endemic plants and fruits, as well as a tincture of the golden root (*Rhodiola Rosea*), an analogue of ginseng growing in Kyrgyzstan, showed good efficacy in neutralization and elimination of OCPs from GT. But further targeted experimental studies on a larger number of patients are required.

Keywords

Organochlorine pesticides, women, breast milk, pathology, therapeutic agents.

Introduction

Persistent organic pollutants (POPs), including the most common and dangerous organochlorine pesticides (OCPs) is a problem that remains pressing. In rural areas, uptake of OCPs into the human body occurs by ingestion in 80-90% of cases, in urban areas - in 100%. In women living in the vicinity of the former pesticide storehouses, agro-airstrips and pesticide dumpsites, OCPs are detected in 100% of cases. OCP exposure is linked to higher rates of various pathologies of pregnant women, fetuses, newborns and infants [1]. In southern Kyrgyzstan, OCPs are the major cause of a number of pathologies, including cancers, hepatitis, infant encephalopathy, dysbacteriosis, immunodeficiency, anemia, intestinal (infection) disorders of unknown etiology, and other pathologies. For the prevention of the above pathologies, taking into account the fact that 90-100% of OCPs are taken into the body through the gastrointestinal tract (GT), there is only one way to eliminate OCPs from the GT and this is a search for therapeutic agents for their elimination from the body. It's well known that pectin substances are used to remove ecotoxicants [2-7] as well as antioxidants - dibunol, emoxipin, cytochrome and other agents inhibit lipid peroxidation, i.e. they can be used in complex therapy of different diseases, including in combination with xenobiotics [8]. Numerous data published both in local and foreign literature indicate that there are no effective therapeutic agents for the neutralization and elimination of OCPs from the human body. Not to mention universal, specific antidotes - there are no antidotes; even primitive ones have not been created for OCPs so far. The aim of this work was therefore to search for therapeutic agents for the neutralization and elimination of pesticides from the human body (gastrointestinal tract); it is necessary to search for highly effective agents, cheap, accessible to all segments of the population.

Materials and methods

Breast milk samples were collected from the women who contacted us about the illness of their children from the "maternity hospital" and the women themselves. All samples were collected with the written consent of the participants after explaining to them the purpose of the study. All studies at the Institute of Medical Problems of the Southern Branch of the National Academy of Sciences of the Kyrgyz Republic are carried out only after obtaining the permission (conclusion) of the ethical commission, as well as with the consent of the study participants indicated in medical cards. The developed medical cards contain all necessary information about the woman in labor, her children and spouse (spouse's profession, possible contact with pesticides), place of birth, ethnicity, weight, height, number of pregnancies, childbirth, stillbirths, miscarriages, abortions, information about nutrition (for example, markets where urban women buy food, or gardens/fields where rural women pick fruits or vegetables, consumption of sour milk (national drinks ayran, zhuurat (goat milk airan) suzmo, kymyz) and meat products [9].

A special attention was given to the cases (and there were most of them) when the examinations were carried out at the request of the examined women (since we covered all the expenses for the examination, further conducting treatment aimed at removing of OCPs from the body of nursing mothers with the use of therapeutic agents we obtained from local endemic plants and their fruits which possess sorbent and detoxic properties [10].

Considering the fact that the most common among the OCPs are α-HCH and β-HCH, DDE, and OCPs detection rate and concentration levels in breast milk depend on the time after childbirth [11], we therefore selected the women who came to us on the 28-30th day after childbirth. All women were of the one ethnic group; Uzbek women mainly consume vegetable products, while Kyrgyz women consume meat and dairy products (the above types of OCPs were detected in breast milk of these women).

All women under observation were allocated into 2 groups. Group I consisted of 27 nursing women and Group II (control group) included 24 women. Women in Group II didn't receive any treatment. In Group I, a concentrate derived from medicinal herbs was used to neutralize and remove pesticides from GT, including crushed seeds which contain unsaturated fatty acids up to 4%, growing in southern region of the Kyrgyz Republic in mid- and high-mountain areas; they are rich in vitamins, pectins, microelements and biologically active substances, with slightly bile, urine and diaphoretic properties. The concentrate was used in the form of tea daily, 2 teaspoons (15 grams) per 300 ml of boiling water, brewed in a thermos and administered during a day [12]. A golden root tincture (analogous to ginseng) (produced by the Institute of Medical Problems, Southern Branch of the National Academy of Sciences of the Kyrgyz Republic) derived from Rhodiola Rosea (1:10 in a 30% alcohol solution) which grows in a high mountainous region (2500-3500 meters above sea level) in Chon-Alay district of the Osh Region of the Kyrgyz Republic. The tincture was administered by 25-30 drops twice a day. The course of treatment was 10-12 days.

Breast milk samples were collected for microbiological and toxicological analysis in accordance with the methodological recommendations of the Central Research Institute of Ecology of the Ministry of Health, MNIIEiM, TSOLIUV, Research Institute of Pediatrics and Pediatric Surgery [9].

Morning anterior (initial) and posterior (residual) portions of breast milk were collected from 82 nursing mothers. Breast milk samples (10 ml) were collected in a sterile disposable tube with a lid and then transported to the laboratory in the same container. Sampling of breast milk, blood (5 ml) and urine (10 ml) for OCPs was carried out in accordance with the methodological recommendation [13] on the gas chromatograph "Tsvet-500 M", (Dzerzhinsk, Russia, 1990, upgraded, with software). The presence of the following pesticides was determined: hexachlorocyclohexane (HCH) (α-, β-, γ-, δ-isomers), dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethane (DDD) and dichlorodiphenylethylene (DDE) isomers, aldrin, dieldrin, and heptachlor.

Statistical analysis of the obtained results was carried out using the methods of variation statistics recommended for biomedical research (https://www.medcalc.org/calc/odds_ratio.php).

Odds Ratio and Relative Risk Calculator (MedCalc software, free online) on a Pentium Core Duo PC. The results were processed using MS Access 2003 and MS Excel 2007 for Windows XP, BIOSTAT.

The results obtained and their discussion

The following types of OCPs were found in breast milk of the women under observation: hexachlorocyclohexane isomers: α-HCH, β-HCH, γ-HCH, dichlorodiphenylethylene (DDE). Other types of OCPs - δ-HCH, DDT, DDD, heptachlor, aldrin and dieldrin were not detected in the examined samples of breast milk samples, i.e. α-, β- and γ-HCH and DDE are the most common OCPs. The number of examined and the proportion of women with OCPs in BM samples by groups are shown in Table 1.

In Group I, of the 27 examined women HCH in BM before and after the treatment was detected in 15, concentration levels were 0.0278±0.0.004 and 0.0135± 0.006 mg/l, respectively, DDE - in 24 (88.9%), concentration level was 0.006238 ± 0.0042, after the treatment - in 18 (6.7%), concentration level 0.0031 ± 0.0006 mg/l, P <0.05.

As shown in Table 1, concentration levels of HCH in BM in Group I after the treatment decreased by half, but the detection rates remained unchanged. The number of detected with DDE decreased from 24 (88.9%) to 15 (55.5%), concentration levels were also decreased by half.

In Group II no changes were registered. In control group, the detection rate of HCH and DDE remained unchanged amounting to 66.7%, and concentration levels decreased by 1.16 times, but by 2.01 times against Group I. This is probably due to the intake of OCPs or with an increase in lactation. Concentration levels of DDE slightly increased from 0.0086±0.003 mg/l, after 10 days to 0.01±0.006 mg/l, P = 0.3947.

Concentration levels of OCPs in blood samples before and after the treatment in control group are shown in Table 2.

TABLE 1. DETECTION RATES AND CONCENTRATION LEVELS OF OCPS IN BREAST MILK SAMPLES BEFORE AND AFTER THE TREATMENT

Group	Type of OCP	Before the treatment				After the treatment				
		Number of samples	Number of detected	%	Concentration level, mg/l	Number of samples	Number of detected	%	Concentration level, mg/l	P
I	HCH	27	15	55.5	0,0278 ±0,0,004	27	15	55,5	0,0135± 0,006	
									2,05 times decrease	
	DDE	27	24	88.9	0,006238± 0,0042	27	18	66,7	0,0031± 0,0006	0.5511
									2,01 times decrease	
II	HCH	24	16	66.7	0,0084± 0,0026	24	16	66,7	0,0072± 0,003	
									1,16 times decrease	
	DDE	24	16	66.7	0,0086± 0,003	24	16	66,7	0,01±0,006	

TABLE 2. OCPS DETECTED IN BLOOD SAMPLES BEFORE AND AFTER THE TREATMENT

Group	Type of OCP	Before the treatment				After the treatment				
		Number of samples	Number of detected	%	Concentration level, mg/l	Number of samples	Number of detected	%	Concentration level, mg/l	P
I	HCH	27	9	33,3	0,026744 ± 0,009934	27	6	22,2	0,0005± 0,000189	0.29
									53,5 times decrease	
	DDE	27	15	55,5	0,00257 ± 0,0006	27	15	55,5	0,0009 ± 0,000063	
									28,5 times decrease	
II	HCH	24	16	66,7	0,0093± 0,0031	24	16	66,7	0,0068± 0,0021	
									1,36 times decrease	
	DDE	24	12	50,0	0,0033± 0,0014	24	16	66,7	0,0038± 0,0013	
									1,15 times increase	

HCH in blood samples was detected in 9-33.3%, concentration level was 0.026744±0.009934 mg/l; after the treatment HCH was found in 6 – 22.2%, P=0.29, concentration level was 0.0005±000189 mg/l or decreased by 53.5 times. DDE detection rate remained at the same level, amounting to 55.5%, but concentration level decreased from 0.00257 ± 0.0006 to 0.0009 ± 0.000063 mg/l or by 28.5 times.

Detection rate of HCH in blood in control group remained unchanged, amounting to 66.7%, but concentration level decreased by 1.36 times, the rate of DDE detection in blood, on the contrary, increased from 50.0% to 66.7% , and concentration– by 1.15 times.

Concentration levels of OCPs in urine samples before and after the treatment (in control group) are given in Table 3.

TABLE 3. OCPS DETECTED IN URINE SAMPLES OF WOMEN BEFORE AND AFTER THE TREATMENT

Group	Type of OCP	Before the treatment				After the treatment				
		Number of samples	Number of detected	%	Concentration level, mg/l	Number of samples	Number of detected	%	Concentration level, mg/l	P
I	HCH	27	3	11,1	0,0002	27	6	22,2	0,0005± 0,00013	0.3544
									58 times decrease	
	DDE	27	9	33,3	0,0029±0,001	27	1	3,7	Traces	
II	HCH	24	12	50,0	0,005±0,0013	24	12	50,0	0,00047 ±0,0002	
									1,06 times decrease	
	DDE	24	3	12,5	0,0002±0,00	24	4	16,7	0,00018±0,00	

Detection rate of HCH in urine samples of women in Group I after the treatment increased from 11.1% to 22.2%, P = 0.3544. Traces of DDE were found in one case after the treatment, P = 0.2051, P = 0.0004, concentration level of HCH decreased by 58 times. In Group II, the detection rate and concentration levels of HCH remained unchanged. Detection rate of DDE in urine samples increased by 4.2%, concentration levels remained unchanged.

Thus, a 10-12 day administration of therapeutic agents produced from local medicinal plants, including endemic herbs and fruits made it was possible to reduce the concentration rates of hexachlorocyclohexane (HCH) - α-, β-, γ isomers and dichlorodiphenylethylene (DDE) in breast milk. Concentration level of HCH decreased by 2.05 times, but the detection rates remained unchanged, DDE - from 88.9% before the start of the treatment up to 66.7%, P = 0.551after the course of treatment. The concentration level decreased by 2.01 times. The level of HCH in blood samples decreased from 33.3% to 22.2%, P= 0.29, concentration decreased by 53.5 times. DDE levels remained at 55.5% even after the course of treatment, but its concentration decreased by 28.5 times.

The level of HCH in urine samples increased from 11.1% before the treatment to 22.2% after the course of treatment, but concentration level decreased by 58 times, DDE - from 33.3% before treatment to 3.7% after the treatment, and only DDE traces were detected, P=0.0004. In control group, the levels HCH and DDE in breast milk samples and HCH level in blood samples remained at 66.7%, in urine samples - remained unchanged. DDE level in breast milk, on the contrary, increased from 50.0% to 66.7%, in urine - the level of HCH remained at 50%, and DDE - increased from 12.5% to 16.7%.

Despite the results obtained, there are still many questions to be answered. First, a question concerning the therapeutic agents derived. These agents are multicomponent, they contain pectin substances with sorbent properties, contain lipids that can absorb OCPs (because OCPs are mostly found in fats - lipotropic), antioxidant vitamins, trace elements and other biologically active substances, which can probably form compounds with OCPs, preventing absorption from the GT and subsequently eliminated from the body via feces. But this issue requires studies of stool samples for OCPs before and after the treatment. On the other hand, we cannot exclude a possible "decay" or 'decomposition" of OCPs in GT. This issue requires more detailed experimental studies.

Thus, therapeutic agents obtained from medicinal plants, including endemic plants and fruits, as well as a tincture of the golden root (*Rhodiola Rosea*) are effective for the neutralization and elimination of OCPs from GT, but further targeted experimental studies are needed on a larger number of patients.

References

1. Toichuev R .M. The effects of pesticide dumpsites Ak-Chabyr and Tash-Baka of Bazar-Korgon and Suzak districts on the population health and the environment of the region. Herald of the Southern Branch of the National Academy of Sciences of the Kyrgyz Republic. 1/2011. pp. 90-91.
2. Abylaeva B.A. Isolation and study of some physical and chemical properties of pectin substances from pomegranate peel (PUNICA GRANATUM) // Fourth International Scientific Ecological Conference "Problems of reclamation of household waste, industrial and agricultural production." Krasnodar, March 2015. pp. 243-246.
3. Buzina, G.V., Kibrik E.D., Parfenenko V.V. Beet pectin production. M., 1974. pp. 1-26.
4. Kochetkova A.A. New aspects of the use of pectin.//Food industry. No. 7. - 1992.
5. State Pharmacopoeia of the Russian Federation. - 12th ed. - M.: Scientific center for expert evaluation of medical products/ 2007. Part 1. 704 p.
6. Onishchenko G.G., Novikov S.M., Rakhmanin Yu.A. Basics of risk assessment of environmental chemical pollutants exposure to public health. M.: NII ECH and GOS, 2002. 408 p.

7. Berezov T.T., Korovkin B.F. Biological chemistry: Textbook. – 3rd ed., M.: OJSC Publishing House "Medicine", 2007. 704 p.
8. Mashkovsky M.D., Therapeutic agents. A guide for doctors. Vol. 2, 13th ed., Tashkent-1998.
9. Bacteriological control of breast milk. Methodological Recommendations. TsNIIE MZ, MNIIEiM, TsOLIUV, Research Institute of Pediatrics and Pediatric Surgery, Moscow, 1984. 34 p.
10. Toichuev R.M., 13-16 September, Barcelona, Spain, No. 597. (2011).
11. Toichuev R.M., Zhilova L.V., Toichueva A.U., Paizyldaev T.R., Khametova M.Sh., Rakhmatillaev A. Organochlorine pesticides in breast milk of urban women in Kyrgyzstan, CHEMICAL SAFETY SCIENCE, 2020, 4, (1), 197 -215.
12.Toichuev R.M., Abdyraeva B.R. Ways of reducing concentrations of organochlorine pesticides in breast milk (certificate) KSMA named after. I.K. Akhunbaev. 01.19.2009. No. 10/09 Reg. No. 10.
13. Guidelines for the selective gas chromatographic determination of organochlorine pesticides in biological media (urine, blood, adipose tissue) and in breast milk. MU No. 3151-84. M., 1990.

OUR EXPERIENCE OF APPLYING THE RESULTS OF RESEARCH AND EVIDENCE-BASED MEDICINE FOR IMPROVING THE AWARENESS, ACHIEVING COMPLIANCE WITH SAFETY MEASURES AND IMPLEMENTING RECOMMENDATIONS BY THE POPULATION LIVING IN THE AREAS POLLUTED BY ORGANOCHLORINE PESTICIDES

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Summary

The paper presents the results of the effectiveness of seminars, lectures, talks, meetings to raise the awareness and knowledge of the Stockholm Convention by using evidence-based medicine in the areas polluted by organochlorine pesticides (OCPs). This approach proved to be effective in raising population awareness regarding OCPs pollution and their health effects. Recommendations for those who are planning to conduct seminars, webinars are as follows: to carry out a comprehensive study of the environment and local population and use the data obtained when conducting seminars and webinars.

Keywords

Awareness, population, the Stockholm Convention, organochlorine pesticides, science, evidence-based medicine.

Introduction

The problems of organochlorine pesticides (OCPs) in the south of Kyrgyzstan are caused by two pesticide burial grounds left since the Soviet era; they are located in the Suzak district of the Jalal-Abad region; 45 sites for agricultural aviation with the residues of pesticides stored there; 183 storehouses where pesticides were previously stored; some territories, the so-called "plague zones", where OCPs were used to fight against fleas of marmots - carriers of the plague; fields where industrial crops of cotton and tobacco used to be grown and where DDT was used for pest control [1]. Despite the fact that pesticides were used there 35 years ago, OCPs are still found in the environment [2] and will be found in the future, while getting into the body through food OCPs cause various pathologies [1,3,4,5,6,7] and also effect the quality of breast milk in lactating women [8,9,10], and food, reducing the content of vitamin C in vegetables and fruits, loss of taste [11]. Therefore, the problem of preserving the health of the population living near these places comes first, at the same time, one of the reasons for the ingestion of OCPs from the environment is the lack of knowledge and awareness of the population about the places where OCPs are found and their impact on human health. Measures taken to eliminate pesticide factors by raising awareness and implementing recommendations based on previously used methodologies did not give the desired result. On the other hand, there are no specific antidotes against OCPs, therefore, in order to preserve the health of the population living near these areas, it became necessary to develop ways to raise awareness and implement evidence-based recommendations to the population.

And accordingly, the objective of the work was to raise the awareness, susceptibility and executive discipline of the population living near these places, to develop evidence-based methods, acceptable, simple recommendations with the elements of evidence-based medicine and evaluate their effectiveness.

Materials and methods

In studying the effects of OCPs on human body, type, concentration and the amount of detected types of pesticides in the body are of great importance, so it became necessary to determine the degree of environmental pollution with organochlorine pesticides and "it is advisable to use breast milk as a biomarker" [12], i.e. breast milk for determining the degree of environmental pollution is optimal and cheap, without any "damage" to the health of the examined women.

To raise the awareness and executive discipline of the population living in environmentally unfavorable places, Institute of Medical Problems (IMP), South Branch of the National Academy of Sciences of the Kyrgyz Republic formed a group of toxicologists, chemists, microbiologists, radiologists, ecologists, epidemiologists, and other clinical, laboratory and diagnostic specialists to develop acceptable, simple, easy to implement and scientifically based methods and recommendations with the elements of evidence-based medicine for the local population. The group studied the effects of OCPs found in breast milk, blood, urine, feces, semen, hair, nails, tumor and "pathological" tissues, organs and in the environment and their effects in the examined women and their newborns and children. Simultaneous examinations were carried out by obstetricians, gynecologists, neonatologists, neuropathologists, cardiologists, endocrinologists, gastroenterologists, hepatologists, hematologists, nephrologists, therapists, dentists, infectious disease specialists, oncologists, immunologists, and other specialists. Previous deliveries of the examined women were analyzed, past and concomitant diseases were also taken into account, full description of the placenta-afterbirth was made, histological, chemical (including neutron activation and mass spectral analysis) and microbiological analyses were performed. Children were also examined by neonatologists, neuropathologists, infectious disease specialists, as well as pediatric surgeons, orthopedists, since some, including neurological pathologies, manifested themselves later [13]. The data obtained were entered in a specially designed research map and Excel sheet. Laboratory specialists: toxicologists, chemists, microbiologists, radiologists collected human and environmental samples for laboratory research, environmental experts studied the presence of active factors, epidemiologists - the disease incidence of the population depending on pesticide "loads" and other environmental factors, clinical specialists carried out preventive examination of the population.

Prior to the start of the research, a number of meetings with the local population, activists, leaders of settlements, deputies of local rural administrations, specialists and members of NGOs was held to explain the goals, objectives and methods of the survey and the necessity of conducting research data. When clinical and laboratory results were obtained, a methodology for seminars, webinars, talks, lectures and preparation of handouts, manuals, etc. for the local population and other professionals was developed. Moreover, during epidemiological, clinical and laboratory studies, meetings with the population were carried out; we explained them how we could help them and what the population and local authorities could do.

The study was conducted in the southern cotton-growing regions of Kyrgyzstan, i.e. in the areas heavily polluted by pesticides used to control cotton, tobacco and plague vectors. The data of the preliminary assessment of soil and water pollution by OCPs in the south of Kyrgyzstan are given in [1], the results of earlier studies and other known data on cases of detection of OCPs in food and their concentrations are also collected there. As a control, the study involved women living in pesticide-free two mountain zones.

To identify the places and degree of OCPs pollution, clinical and epidemiological studies were performed. We also analyzed OCPs concentrations in breast milk of lactating women.

Breast milk (BM) of 125 women, aged 18 to 45 years, with children from 6-10 days old, living in different zones, who currently do not have direct contact with pesticides, were subjected to examination. Depending on pesticide pollution, all the examined were divided into 5 groups and subgroups a) and b) depending on the source of OCPs. Group I included 20 women living in a conditionally pesticide free mountain zone in the Alai region. This zone (300 thousand hectares) is a conditionally pesticide free zone, because there were some plague foci (treated by pesticides) were registered in the past. The local population consume local food products of plant and animal origin.

Subgroup a) included 3 women living in the village of Bulolu, located near the former plague foci, where, DDT was used (until 1982) to treat marmot burrows against fleas as plague carriers. During all these years, domestic animals grazed and gave birth near these places. As a vegetable food, the population used their own garden. Subgroup b) included 17 women living far from the former plague foci at a distance of 4–7 km, where animals grazed 8–10 km from the former plague foci.

Group II included 32 women living in pesticide free mountainous area in the Kara-Kulzha district. Of them, subgroup a) included 16 women who used local food products, and subgroup b) consisted of 16 women who consumed food from the central bazaar.

Group III includes 17 women living in the Aravan formerly cotton-growing region. The population grow cotton and are engaged in cultivation of vegetables, melons, legumes, fodder crops.

Group IV included 24 women living in the cotton-growing zone, in the village of Burgandy, Nooken district, Jalal-Abad region. Of them, subgroup a) included 9 women living in the village of Uuru-Zhar, located 1–2 km from the unpaved agricultural airport, which functioned until 1985, then it was turned into an arable field, where cotton was grown until 1994, the population switched to cultivation of fruits, vegetables, gourds, fodder crops. In this area, the surface of the earth has slopes, i.e. the soil and OCPs are washed away by irrigation water and rainfalls. Subgroup b) included 24 women living in villages located at a distance of 4 to 6 km above the agro-airport.

Group V consisted of 32 women living in the cotton-growing zone of the same district in the rural community of Sakaldy, where until 1989 a stationary agro-airport and pesticide storehouses functioned. Currently, the agro-airport and pesticide storehouses (Fig. 1) are destroyed and abandoned. The terrain is flat, rainwater is deposited, irrigation water partially washes away the soil, including OCPs. Subgroup a) included 15 women living in a settlement located at a distance of 2–3 km from the agro-airport and near destroyed pesticide storehouses. Cotton and vegetables, fruits, fodder crops are grown here. Subgroup b) included 17 women living in the same rural community in the village of Arimzhan, located 5–6 km from the airport and pesticide storehouse.

Breast milk samples for microbiological and toxicological studies were collected according to the methodological recommendations of the Central Research Institute of Ecology of the Ministry of Health, MNIIEiM, TSOLIUV, Research Institute of Pediatrics and Pediatric Surgery [14]. For analysis, morning anterior (initial) and posterior (residual) portions of breast milk were collected from 125 lactating women, BM was collected in an amount of 10 ml in a sterile disposable tube with a lid, delivered to the toxicological laboratory in a container within 130 to 180 minutes.

Organochlorine pesticides in BM was detected in accordance with the methodological recommendation [15] on a gas chromatograph "Tsvet-800 M", (Dzerzhinsk, Russia, 1990, upgraded, with software). The presence of the following pesticides was determined: hexachlorocyclohexane (HCH) (α-, β-, γ-, δ-HCH isomers), dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethane (DDD) and dichlorodiphenylethylene (DDE) isomers, aldrin, dieldrin, and heptachlor.

Statistical processing of the obtained results was carried out using the methods of variation statistics recommended for biomedical research from the site (https://www.medcalc.org/calc/odds_ratio.php).

In this study, the following types of OCPs were found in BM of the examined women: hexachlorocyclohexane isomers: α-HCH, γ-HCH, β-HCH, DDE. The highest percentage (100%) and degree of OCPs pollution was found in Group V subgroup a) – women living in the village Sakaldy, near the former agricultural airport, the smallest percentage in Group I subgroup b) far from the former plague foci - 5.88%, the concentration level was 0.0007 ± 0.00 mg/l in women who lived far away (at a distance of 6–7 km) from the former plague foci and consumed food grown in their garden and obtained from domestic animals grazing away from the former plague foci, i.e. away from the areas polluted by OCPs. Therefore, the data obtained indirectly indicate that, despite the fact that more than 30 years have passed, OCPs remained unchanged in the soil. The highest concentration was found in Group I subgroup a) in women living near former plague foci in 2 women, the concentration was 0.29 ± 0.02 mg/l, but it was not reliable due to the small amount, while in Group V in subgroup a) in women living near the destroyed agro-airstrip and pesticide storehouses that functioned before 1989, the total concentration of OCPs was 0.044877 ± 0.012 mg/l, P=0.01. In some women, up to 4 types of OCPs were simultaneously detected in BM. Among the mothers and children, the incidence rate was higher in women when 3-4 types of OCPs were detected in BM and concentrations over 0.03 revealed breast pathologies, menstrual irregularities, latent forms of toxic hepatitis, impaired intestinal biocenosis and other pathologies; when aldrin and heptachlor were detected, regardless of their concentration, the same pathologies with clinical manifestations were diagnosed. The disease incidence was higher in children than in mothers, it was manifested by pathologies of the gastrointestinal tract, jaundice-hepatitis, encephalopathies, malnutrition, immunodeficiency, congenital and other pathologies, impaired absorption of milk, repeated incidence associated with the intake of OCPs through the mother's breast milk was reported [12].

When conducting seminars, webinars, meetings, conversations and preparing calendars, booklets, handouts, we used the results of scientific research, data on pathologies diagnosed by medical specialists and laboratory tests. We also prepared recommendations for the local population, local authorities, agronomists, environmentalists, medical workers, members of different NGOs and activists.

Nevertheless, based on the results of the study, the following recommendations can be made for the population living in the study area:

− prohibit farmers from growing fruits, vegetables, legumes, gourds, fodder crops, as well as grazing animals at a distance of 4-5 km from these objects in the places of former agro-airports and destroyed pesticide storehouses;

− state environmental protection agency and local agronomists are recommended to carry out soil remediation through winter-spring soil leaching, planting trees, which are then used by local residents only as building material;

− for medical workers, the population living near the places of former agro-airports and pesticide storehouses to be classified as a risk group and for women to conduct BM examinations for OCPs, if they are found, to take appropriate preventive measures;

- as regards scientific aspect, it is necessary to study the actions of OCPs in the human body that penetrate through the gastrointestinal tract, taking into account the nature of the diet and lifestyle of the local population, to search for funds based on local raw materials to eliminate OCPs from the gastrointestinal tract;

Also, during the seminars, we cited specific examples (for example, your and your child's illness is due to OCPs in your body, which came through cow's milk, meat and dairy, vegetables, melons, grains and legumes grown in pesticide polluted soils and waters - places of former pesticide storehouses, agro-airports, fields of former cotton and tobacco fields. Therefore, in order to maintain health, you should follow these recommendations, in these places and fields you have to plant trees for building materials, graze animals away from these places). Such meetings and seminars gave good results, the local population began to comply with our recommendations and demand that the authorities solve environmental problems.

Thus, in order to improve, raise the awareness and achieve compliance with safety measures and implementation of recommendations by the population living in places polluted by organochlorine pesticides, it is necessary to conduct a comprehensive study to determine the "degree of danger" for the pollution health. For this it is necessary to use breast milk as a biomarker and carry out a preventive examination of the population. When carrying out activities, use the data obtained from studies with specific examples, provide the local population the results of studies and recommendations so that they could demand from the local authorities the implementation of measures to eliminate OCPs pollution on the basis of the Stockholm Convention [16]. Scientifically based approach with the elements of evidence-based medicine will make it possible to raise the awareness of the population; their demand will also be justified, i.e. the population will demand from the authorities to withdraw the residues of obsolete pesticides and toxic substances, demand compensation for the “damage” caused to their health.

To increase the effectiveness of ongoing seminars, meetings among the population and to raise the awareness and executive discipline, it is recommended to conduct a comprehensive study of the area, examine the local population by using our methods with the elements of evidence-based medicine.

References

1.Toichuev R.M., Zhilova L.V., Makambaeva G.B., Payzildaev T.R., Pronk W., Bouwknegt M., & Weber R. (2018). Assessment and review of organochlorine pesticide pollution in Kyrgyzstan. Environmental Science and Pollution Research, 25 (32), 31836-31847. https://doi.org/10.1007/s11356-017-0001-7.

2. Toichuev R.M., Toichueva A.U., Monitoring of organochlorine pesticides in breast milk of women in Kyrgyzstan. Chemical Safety, 2019, Vol.3, No. 2, pp. 94-109.

3. Toichuev R.M., Zhilova L.V., Payzildaev T.R. et al. Organochlorine pesticides in placenta in Kyrgyzstan and the effect on pregnancy, childbirth, and newborn health // Environmental Science and Pollution Research. 2018. V. 25. No. 32. P. 31885.

4. Malevich Yu.K., Simonova E.V. Organochlorine pesticides and their influence on the qualitative composition of breast milk of puerperas // Belarusian Medical Journal. 2003. No. 3. P. 86.

5. Toguzbayeva K.K., Niyazbekova L.S., Seyduanova L.B. et al. Influence of ecological factor on the state of health of the rural population // International Journal of Applied and Fundamental Research. 2016. No. 4-2. P. 507.

6. Karakushikova A.S., Toguzbayeva K.K., Myrzakhmetova Sh.K. Evaluation of the influence of the environmental factor on the state of health of the rural population // Bulletin of KazNMU. 2014. No. 3(3). P. 23.

7. Tadevosyan N.S. Monitoring of individual organochlorine pesticides in the body of rural women in Armenia and indicators of the physical development of newborns and children of the first year of life // Bulletin of KSMA named after I.K. Akhunbaev. 2016. No. 5. P. 159.

8. Hedley A.J., Hui L.L., Kypke K. et al. Residues of persistent organic pollutants (POPs) in human milk in Hong Kong // Chemosphere. 2010. V. 79. No. 3. P. 259.

9. Madykova J.A. Contamination of cow’s milk with organochlorine pesticides in the Osh Province, Kyrgyzstan // Proceedings of 29th Annual Sci. Conf. International Society of Environmental Epidemiology (ISEE 2017). Sydney, Australia, 2017.

10. Toichuev R.M. Adverse effects of pesticide deposit sites Ak-Chabyr and Tash-Baka located in Bazar-Korgon and Suzak districts of Zhalal-Abad region (Kyrgyz Republic) on the population health an biota // Proceedings of the 11th HCH and Pesticides International forum, 7-9 September 2011, Azerbaijan.

11. Shtenberg A.I. Residual content of pesticides in food. M.: Medicine, 1973. P. 181.

12. Toichueva A.U., Sakibaev K.Sh., Payzildaev T.R., Zhilova L.V., Toichuev R.M. Breast milk as a biological marker for determining the pollution of the environment with organochlorine pesticides in southern Kyrgyzstan. Chemical Safety. 2021. V. 5. No. 1. PP. 215-236.

13. Toichuev R.M., Zhilova L.V., Toichueva A.U., Payzildaev T. R., Khametova M.Sh., Rakhmatillaev A. Organochlorine pesticides in the breast milk of urban women of Kyrgyzstan. Chemical safety. 2020. V.4. No. 1. PP. 197-215.

14. Bacteriological control of breast milk. Method. rec. TsNIIE MZ, MNIIEiM, TSOLIUV, Research Institute of Pediatrics and Pediatric Surgery. M., 1984.

15. Guidelines for selective gas chromatographic determination of organochlorine pesticides in biological media (urine, blood, adipose tissue) and breast milk. MU No. 3151-84. M., 1990.

16.http://www.un.org/ru/documents/decl_conv/conventions/pdf/pollutants.pdf Stockholm. Convention on Persistent Organic Pollutants. http://www.un.org/ru/documents/decl_conv/conventions/pdf/pollutants.pdf (accessed: 10/30/2019).