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Pesticide Mixtures *in Vitro* Assessment and Toxicological Prioritization at Concentrations from Occupational Scenarios

John Godleski*

Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts, USA

Introduction

Pesticide residues are present in humans on a regular basis, with agricultural workers being particularly vulnerable. The cumulative risk evaluation of pesticide combinations is a significant topic in this setting. At field concentrations, the toxicological profiles of thirteen pesticide mixes used for grapevine protection, comprising ten active chemicals .Pesticides are man-made chemicals used to protect agricultural crops against pests such as insects, fungus and undesired plants (weeds); they include insecticides, herbicides, fungicides and rodenticides. Pesticides, despite their carefully controlled use, can be widely disseminated in the environment, potentially causing harm to humans. Indeed, multiple studies have documented the presence of their residues in a range of matrices such as water, soil and food, as well as outdoor and indoor air and house dust, demonstrating that exposure is broad and comes from a variety of sources.

Description

Agricultural workers are a subgroup with increased exposure, mostly through inhalation and skin contact. A recent analysis of health risks found an elevated risk of some cancer types in this demographic group, with evidence of DNA damage, oxidative stress and metabolic changes.

It has also been stated that there is an increased risk of neurological illnesses such as Parkinson's disease. Furthermore, thyroid derangements are more frequently connected with occupational exposure than with non-occupationally exposed persons. Importantly, people are simultaneously exposed to many pesticides, either directly or as residues, in various combinations, the consequences of which are still little understood [1]. Because of the importance of this issue for human risk assessment, the European Commission (EC) and the European Food Safety Authority (EFSA) recently published an action plan to accelerate the development of methods for pesticide cumulative risk assessment (CRA) for dietary and non-dietary exposures.

In this context, the current study's goal is to evaluate and prioritise the toxicity of pesticide mixes used in the field by agricultural workers as a case study. Italian agronomists from the Italian autonomous Province of Trento identified thirteen distinct combinations used to treat and protect vineyards against seasonal fungal and mould infestations. Sulfur was utilised as a common component in most of the mixes, as was potassium phosphonate, which was used in substitution or combination with sulphur and the fungicides metrafenone, cyflufenamid, quinoxyfen, folpet, penconazole, dimethomorph, mancozeb and zoxamide [2].

Human liver (HepG2) and lung (A549) cell lines were utilised since they are metabolically competent and represent the primary target organs of the general population as well as occupational exposure. Benchmark Doses were determined for dose-response curves since they were more important for risk assessment. The whole data was then combined using the ToxPi programme to rank and prioritise combination toxicity and display commonalities among the 13 toxicological profiles.

In a one-year trial, DIM demonstrated low damage in the liver, testes and prostate of dogs, according to an EFSA report. DIM lowered cell viability of mouse cortical neurons *in vitro* but had no impact on MMP; consequently, it is fair to speculate that DIM had an additive effect with QUI on cytotoxicity, increasing apoptosis. Among the endpoints evaluated in this study, the decrease in ROS intracellular levels exerted by the majority of combinations is the most fascinating to be further and more thoroughly examined, indicating an autophagy mechanism [3].

These findings clearly show that the toxicological assessment of pesticide mixtures used in the field—representing a real exposure scenario—can be accomplished even when only a limited amount of information on the single active compounds is available, because the ToxPI tool, by integrating multiple sources of data and reducing variables to a single dimensionless score, is capable of capturing minor differences between similar mixtures. Such an approach could easily be extended to other real-life mixtures, with a greater number of endpoints and cellular models implied, to provide robust data for cumulative risk assessment and mixture prioritisation, particularly in occupational contexts, to guide the selection of mixtures with a higher safety margin of exposure [4,5].

Conclusion

By comparing their toxicological profiles and rating their scores, the current technique showed to be a beneficial tool for supporting the prioritising of commercial chemical mixes or mixtures for which not all of the components are entirely described. The findings from the battery of *in vitro* experiments, which were put into the ToxPI tool, revealed that mixes including MAN were the most hazardous, giving more proof for MET's toxicity. Differences across comparable combinations were also detected, bolstering the approach's dependability, which might be expanded to include additional and varied endpoints.

Conflict of Interest

No potential conflict of interest was reported by the authors.

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***Address for Correspondence:** John Godleski, Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts, USA; E-mail: johngodleko@gmail.com*

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