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Toxic effects of pesticide mixtures at a molecular level: Their relevance to human health

Antonio F. Hernández[a,*], Tesifón Parrón[b,c], Aristidis M. Tsatsakis[d], Mar Requena[b], Raquel Alarcón[c], Olga López-Guarnido[a]

[a] Department of Legal Medicine and Toxicology, University of Granada School of Medicine, Granada, Spain
[b] Council of Health at Almeria province, Almeria, Spain
[c] Department of Neuroscience and Health Sciences, University of Almeria, Almeria, Spain
[d] Department of Forensic Sciences and Toxicology, Medical School, University of Crete, Heraklion, Greece

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ABSTRACT

Pesticides almost always occur in mixtures with other ones. The toxicological effects of low-dose pesticide mixtures on the human health are largely unknown, although there are growing concerns about their safety. The combined toxicological effects of two or more components of a pesticide mixture can take one of three forms: independent, dose addition or interaction. Not all mixtures of pesticides with similar chemical structures produce additive effects; thus, if they act on multiple sites their mixtures may produce different toxic effects. The additive approach also fails when evaluating mixtures that involve a secondary chemical that changes the toxicokinetics of the pesticide as a result of its increased activation or decreased detoxification, which is followed by an enhanced or reduced toxicity, respectively. This review addresses a number of toxicological interactions of pesticide mixtures at a molecular level. Examples of such interactions include the postulated mechanisms for the potentiation of pyrethroid, carbaryl and triazine herbicides toxicity by organophosphates; how the toxicity of some organophosphates can be potentiated by other organophosphates or by previous exposure to organochlorines; the synergism between pyrethroid and carbamate compounds and the antagonism between triazine herbicides and prochloraz. Particular interactions are also addressed, such as those of pesticides acting as endocrine disruptors, the cumulative toxicity of organophosphates and organochlorines resulting in estrogenic effects and the promotion of organophosphate-induced delayed polyneuropathy.

1. Introduction

Pesticides are unique, intrinsically toxic chemicals designed to be deliberately spread into the environment to kill off pests. They are comprised of many different categories of chemicals whose toxicity is increasingly reduced as less toxic compounds benefit from stricter regulations. Approximately 5.2 billion pounds were used worldwide in 2006 and a similar amount in 2007 (US-EPA, 2011), but only 1% of this amount reaches the target pests at lethal doses (Gavrilescu, 2005). Herbicides account for the largest portion of that amount, followed by other pesticides, insecticides and fungicides (US-EPA, 2011).

Exposure to pesticides can occur through multiple pathways (e.g. food, drinking water, residential, occupational) and routes (oral, inhalation, dermal). Although the contribution of a given route or pathway to overall exposure depends on the pesticide, it is the totality of exposure, by multiple routes and multiple pathways, what determines the risk (EFSA, 2008). The type and severity of adverse health effects of pesticides are determined by the individual chemical category, the dose and the duration of exposure and the exposure route.

Because of their intrinsic toxicity and limited species selectivity, pesticides exhibit undesirable harmful effects on sensitive non-target organisms such as humans and wildlife populations (Hernández et al., 2011a). Given that humans are much larger than the target species for pesticides, they are expected to be unaffected by small amounts of these compounds. However, pesticides are indeed toxic to humans not only at high doses, responsible for acute poisonings, but even in low doses, as are mixtures of pesticides (Tsatsakis et al., 2009; Zeliger, 2011). Long-term exposures may lead to an array of health effects including cancer and neurodegenerative diseases (Bassil et al., 2007; Kanavouras et al., 2011; Parrón et al., 2011), reproductive and developmental toxicity (Hanke and Jurewicz, 2004) and respiratory effects (Hernández et al., 2011b). It

* Corresponding author at: Department of Legal Medicine and Toxicology, University of Granada School of Medicine, Avda. Madrid, 11, 18071 Granada, Spain. Fax: +34 958246107.
E-mail address: ajerez@ugr.es (A.F. Hernández).

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is now well established that exposure to pesticides during critical periods of development can present lasting adverse effects in early development and later in life, particularly the developing brain and the endocrine system are both very sensitive targets (London et al., 2012).

Since pesticides are often applied in mixtures to crops, their residues can be found in foods and drinking water. However, mixtures of pesticides are common not only in the human food supply but also in the aquatic environment, including surface waters that support aquatic life (Laetz et al., 2009). In fact, more than 50% of all streams tested in the United States contained five or more pesticides (Zeliger, 2011). If streams and groundwaters are used as a source of drinking water, and the previous treatment fails to eliminate pesticide residues, humans can be exposed to mixtures of pesticides and their degradates.

Despite many papers describe the toxic effects of pesticide mixtures, relatively little information is available on the nature of interactions that may occur between the constituents of a pesticide mixture, especially when they deviate from additivity.

2. Toxicological interactions of pesticides

Pesticide interactions include agent-to-agent interactions, toxicokinetic and toxicodynamic interactions. Thus, to make science-based judgments about these interactions it is necessary to have a good understanding of the chemical reactivity, the toxicokinetics (including metabolic pathways) and the mechanisms of action of each compound (IGHRC, 2009).

Exposure to multiple pesticides may cause changes in the toxicokinetics of the individual compounds, thus modifying the predicted toxicity. Toxicokinetic interactions are the result of one pesticide altering the absorption, distribution, metabolism or elimination of others (Reffstrup et al., 2010) and can occur at all dose levels, but the effects may not be measurable at low doses. The most likely effect of these interactions is to alter the relationship between the external dose and the corresponding level of a pesticide at its target site, leading to an alteration in the threshold for effects (IGHRC, 2009).

Toxicodynamic interactions requires that a sufficient amount of a pesticide reach a target tissue and causes some perturbation of normal physiology and that a sufficient amount of a second toxicant also reach the same tissue and causes a second perturbation which either exacerbates (potentiates) or compensates for (antagonizing) the effects of the first compound. The key requirement for toxicodynamic interactions is that the dose levels for each pesticide are sufficient to have an effect (i.e., above the threshold of effect). When dose levels are below thresholds of effect, no toxicodynamic interactions are expected to arise (IGHRC, 2009).

There are two main principles describing how individual pesticides in a mixture affect one another: the concept of additivity and interaction. Additivity expectations can be derived from the concepts of dose addition and independent action, which assume that chemicals act by the same or different modes of action, respectively (Silins and Högberg, 2011). In this situation, also termed "non-interaction", the toxicity of a mixture resembles the effects expected to occur when all mixture components act without enhancing or diminishing their effects. By contrast, interaction arises when the observed mixture effects deviate from what was expected. In this case, one or several pesticides may have interacted with each other, e.g. by facilitating or diminishing their uptake, transport, metabolism or excretion. Interaction is the term for synergisms (mixture effects greater than expected) and antagonisms (Kortenkamp et al., 2009).

The combined toxicological effects of two or more components of a pesticide mixture can take one of three forms: independent, dose addition or interaction.

2.1. Independent action

Independent action, also referred to as response or effect addition, occurs when the toxicological effects of the individual pesticides in a mixture have different mechanisms or modes of action. The effects of such a combination will be the sum of the effects of each compound when given alone, reflecting that they do not influence each other's action, and therefore neither additivity nor potentiating interactions are generally found (Boobis et al., 2008; COT, 2002). When this is applied to pesticide residues that have different modes of action, and each component is below any threshold of effect, the default assumption is that the combined exposure will not have any toxicological effect in consumers, given that the individual exposures do not exceed the respective reference values (Boobis et al., 2008).

2.2. Dose-addition

The generally held view is that dose addition should be assumed for mixtures of pesticides that produce the same toxic effect by affecting the same target organ via the same mechanism of toxicity over the whole dose range (COT, 2002; IGHRC, 2009). Two pesticides act via a common mechanism of toxicity if they cause the same critical effect and act on the same molecular target or tissue or on the same biochemical mechanism of action, possibly sharing a common toxic intermediate (US-EPA, 1999).

The combined effect of pesticides that have similar chemical structures and/or modes of toxic action can be predicted by an additive toxicity approach that assumes that the cumulative toxicity of the mixture can be estimated from the sum of the individual toxic potencies of each individual compound (Lydy et al., 2004; Hernández et al., 2011a). This is the case of organophosphates (OP) pesticides, (di)thiocarbamates or chloroacetanilides. In concurrent multiple OP exposure, a summation of the inhibitory effects of individual compounds on acetylcholinesterase (AChE) activity is usually observed. This also applies to OPs and *N*-methylcarbamates (NMC), two different classes of insecticides that share a common mode of toxic action: inhibition of AChE. According to acute oral toxicity (LD_{50}), the combined effects of two insecticides have been reported to be additive for OPs plus OPs and for OPs plus NMC in most cases (Sun et al., 2000).

Additive effects may be observed when a mixture of two pesticides, each below the no observed effect, produces a predicted toxic effect when the sum of their concentrations is greater than the threshold level for toxic action (Zeliger, 2011). Given that food residues of pesticides are generally found at exposure levels far below their respective NOAEL, they are not expected to cause more than an additive effect.

2.3. Interaction

Chemical interactions represent a deviation from simple additivity because individual compounds affect toxicity of one another, resulting in more or less than an additive effect (Rider and LeBlanc, 2005; Silins and Högberg, 2011). Thus, the combined effects of two or more pesticides is either greater (supra-additive, potentiating, synergistic) or less (infraadditive, inhibitive, antagonistic) than that predicted on the basis of dose-addition or response-addition (Boobis et al., 2008). Interaction does not occur at doses that are at or below the NOAEL of pesticides in a mixture; however, when exposures exceed their respective NOAELs, both toxicodynamic and