In vitro bioassays reveal that additives are significant contributors to the toxicity of commercial household pesticides.

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Highlights

- *In vitro* bioassays used to assess toxicity of commercial pesticides vs active ingredients
- Additives in commercial pesticides significantly contributing to the toxicity of commercial pesticides
- Human health and environmental risk assessments that do not include additives are underestimating the risks of pesticides

Abstract

Pesticides commonly used around households can contain additives of unknown concentrations and toxicity. Given the likelihood of these chemicals washing into urban waterways, it is important to understand the effects that these additives may have on aquatic organisms. The aim of this study was to compare the toxicity of commercially available household pesticides to that of the active ingredient(s) alone. The toxicity of five household pesticides (three herbicides and two insecticides) was investigated using a bacterial luminescence bioassay and an algal photosynthesis bioassay. The commercial products were up to an order of magnitude more toxic than the active ingredient(s) alone. In addition, two commercial products with the same listed active ingredients in the same ratio had a 600× difference in potency. These results clearly demonstrate that additives in commercial formulations are significant contributors to the toxicity of household pesticides. The toxicity of pesticides in aquatic systems is therefore likely underestimated by conventional chemical monitoring and risk assessment when only the active ingredients are considered. Regulators and customers should require more clarity from pesticide manufacturers about the nature and concentrations of not only the active ingredients, but also additives used in commercial formulations. In addition, monitoring programmes and chemical risk assessments schemes should develop a structured approach to assessing the toxic effects of commercial formulations, including additives, rather than simply those of the listed active ingredients.

Keywords: herbicides, insecticides, algae toxicity, bacteria toxicity, commercial pesticide mixtures, additives/adjuvants

Introduction

Pesticides are used globally for the control of unwanted pests in agriculture, aquaculture, horticulture, and also for various general household applications. A range of products exists for targeting specific pests, such as insecticides, rodenticides, herbicides and fungicides (Alavanja, 2009). There are many benefits to pesticide use, most notably, more efficient food production and the control of disease vectors such as mosquitoes (Cooper and Dobson, 2007). Despite the benefits of pesticides, the potential for effects in non-target species is a topic of international relevance, with implications for human and environmental health. As such, the production and use of pesticides are generally regulated by governments and regulatory agencies. In Australia, pesticide regulation up to the point of retail sale occurs under the National Registration Scheme (NRS), administered by the Australian Pesticides and Veterinary Medicines Authority, while control of use is regulated by various state and territory legislations (King et al., 2013). However, environmental monitoring, and therefore risk assessments, are generally limited to the active ingredients and known additives of pesticide formulations.

Commercially available pesticide formulations are labelled with the identity and concentration of the active ingredients; the chemicals of known potency and mode of action used to elicit the desired effect. However, active ingredients are usually combined with additives that enhance their efficacy (*e.g.*, surfactants, attractants/repellents, stabilisers, dyes, fertilizers, synergists), which can comprise up to 99% of the formulation, but are often not disclosed (Mullin et al., 2016). Additives have various economic and environmental benefits, primarily reducing the amount of active ingredient required to achieve the desired effect. However, the United States Environmental Protection Agency (USEPA) estimates that there are over 4,000 additives used in commercially available pesticide formulations, with 374 of these designated as ‘toxic’ under U.S. federal laws (Weinhold, 2010). For many of the

remaining additives, the toxicity remains unknown (Cox and Surgan, 2006; Weinhold, 2010). Since pesticides commonly find their way into aquatic environments after use through runoff, it is important that we strive to fully understand the toxicity of commercial pesticide formulations, including that of both active ingredients and additives.

There is mounting evidence that additives included in commercial pesticide formulations can modulate the toxicity in exposed organisms (Surgan et al., 2010; Mesnage and Antoniou, 2018). In particular, the toxicity of glyphosate and its commercial products (*e.g.*, Roundup®, Zero®) has been extensively studied (*e.g.*, Cuhra et al., 2016; Myers et al., 2016; Tarazona, 2017), and the general consensus is that additives present in commercial mixtures significantly increase glyphosate toxicity (Mesnage et al., 2015; Richard et al., 2005; Vincent and Davidson, 2015). Other (non-glyphosate) domestic pesticide formulations have also been shown to be more toxic to aquatic organisms such as fathead minnows (*Pimephales promelas*), water fleas (*Ceriodaphnia dubia*) and frog (*Rana clamitans*) tadpoles, compared to the active ingredients alone (Beggel et al., 2010; Chen et al., 2010; Puglis et al. 2011). However, other common household pesticides have been studied to a lesser extent, and knowledge gaps therefore exist regarding mixture toxicity between active ingredients and additives in commercial formulations. Considering their widespread usage, proprietary shielding from disclosure, and multiple lines of evidence suggesting increased toxicity in non-target organisms, commercial additives are clearly an important topic globally, and one that warrants increased research and regulatory attention.

Component based-approaches using the mixture toxicity models of concentration addition (CA) and independent action (IA) have been suggested as part of a tiered process to evaluate the toxicity of household products (*e.g.*, Backhaus et al. 2013). The toxicity of chemicals with a common mode of action usually closely follows CA predictions, while chemicals with different modes of action act according to IA, with both models assuming no interaction

between the mixture components in the toxicokinetic and toxicodynamic phases (Backhaus and Faust, 2012). Using available literature data for a number of species, Coors and Frische (2011) evaluated the suitability of the CA mixture toxicity model to predict the toxicity of commercial combination products using individual active ingredients effect data. Based on the model deviation ratio, over 50% of CA predictions were found to be within a factor of two of the observed toxicity. Further, Belden et al (2007) found that 88% of CA predictions were within a factor of two of the observed values for pesticide mixtures. The accuracy of the mixture toxicity model prediction can be affected by the quality of the individual component effect data and by not considering relevant, but potentially unknown, individual components in the model (Backhaus et al. 2013).

In Australia, common household pesticide formulations (other than those containing glyphosate) include active ingredients such as simazine, 3-amino-1,2,4-triazole (amitrol), pyrethrum (a natural plant extract), 2-methyl-4-chlorophenoxyacetic acid (MCPA), 3,6-dichloro-2-methoxybenzoic acid (dicamba) and diazinon (Table 1). Several of these chemicals have been detected in aquatic waterways (Bailey et al., 2000; Davis et al., 2008; Rippy et al., 2017), although monitoring data is patchy. Two of the main disclosed additives in household pesticides include piperonyl butoxide, a synergist that increases the potency of insecticides and herbicides (Varsano et al., 1992), and ammonium thiocyanate, a herbicide additive that acts as a synergist. Such additives are rarely monitored in environmental samples, despite the fact that their inclusion in household pesticide formulations suggests that they may also ultimately end up in aquatic receiving environments.

In vitro bioassays offer a valuable testing method in environmental toxicology, providing a low cost, high throughout and repeatable test platform for a variety of specific and non-specific toxicological endpoints (Escher and Leusch, 2012). When they include unicellular organisms that are ubiquitous in aquatic environments (*e.g.*, bacteria and algae), *in vitro*

bioassays can also provide important ecological information for receiving waterways. *In vitro* bioassays are thus particularly well suited for large-scale screening of countless contaminant and mixture scenarios (Escher and Leusch, 2012; Ilboudo et al., 2014). Despite these strengths and obvious capacity for exploring the effect of chemical mixtures, there are few studies applying *in vitro* bioassays to characterise pesticide-additives interactions (*e.g.*, Coalova et al., 2014; Fine et al., 2016; Zahn et al., 2018). The objective of this study was to compare the toxicity of laboratory-grade active ingredients and additives commonly found in household pesticides to the toxicity of the commercially available products, using two well established *in vitro* bioassays – inhibition of luminescence in bacteria (cytotoxicity) and inhibition of photosynthesis and non-specific toxicity in green microalgae.

Methods

Reagents and preparation of stocks

Five commonly available household pesticides were purchased from a hardware store. These included three herbicides ("Path Weeder", "Path and Patio Weeder" and "Bindii and Clover Weeder", hereafter referred to as CO_H1a, CO_H1b and CO_H2, respectively) and two insecticides ("Pyrethrum Insect Pest Killer" and "Crawly Cruncher", hereafter referred to as CO_I1 and CO_I2, respectively). All listed ingredients in these products were purchased as high purity laboratory-grade chemicals from Sigma-Aldrich (Australia): simazine (CASRN: 122-34-9; 99.9% purity), amitrol (CASRN: 61-82-5; 99.9%), ammonium thiocyanate (CASRN: 1762-95-4; 97.5%), MCPA (CASRN: 94-74-6; 99.9%), dicamba (CASRN: 1918-00-9; 99.9%), pyrethrum extract (CASRN: 8003-347; 52.2%), piperonyl butoxide (CASRN: 51-03-6; 99.9%), diazinon (CASRN: 333-41-5; 98.5%). Stocks of the active ingredients were prepared in methanol (CASRN: 67-56-1; ≥99.9% purity), and artificial mixtures (AI_H1, AI_H2, AI_I1 and AI_I2) replicating the advertised ingredients of the household pesticide formulations were prepared by combining appropriate volumes of the active ingredients

stocks (Table 1). A single artificial mixture was prepared for CO_H1a and CO_H1b as these both contained the same active ingredients in the same ratios. The maximum solubility of pure ingredients in methanol was often lower than the concentrations reached in the household products. In these cases, the maximum concentration of the least soluble active ingredient (in methanol) was used, and the ratios of the other active ingredients were adjusted accordingly to represent the artificial mixtures.

All concentrations were converted to molar units so that more direct comparisons could be made in the bioassay results. For mixtures, the molar concentration was calculated by summing the molar concentration of all ingredients listed in each household product. The concentration ranges for each chemical and mixture tested in the bioassays are provided in Figures 1 and 2.

Bioassay analyses

The toxicity of all household products, individual active ingredients and artificial mixtures of active ingredients was analysed using two *in vitro* bioassays: 1) the bacterial luminescence toxicity screen (BLT-Screen; van de Merwe and Leusch 2015) and 2) the algal toxicity test by imaging pulse amplitude modulated fluorometry (algal IPAM; Escher et al., 2008). The BLT-Screen measures non-specific toxicity, and is therefore useful for assessing the relative toxicity of chemicals with a wide range of modes of action, as is the case here. The algal IPAM bioassay measures both specific (inhibition of photosynthesis) and non-specific (phytotoxicity) effects, and is therefore particularly useful for assessing the toxicity of herbicides.

The bacterial luminescence toxicity screen (BLT-Screen) methods have been previously described by van de Merwe and Leusch (2015). Briefly, the household products, active ingredients and artificial mixtures were added to a phosphate-buffered saline medium and

serially diluted in a 96-well plate. Naturally luminescent bacteria, *Photobacterium leiognathi*, were then added to each well (from a cryopreserved stock), and the luminescence of each well was measured in a Fluostar plate reader (BMG Labtech, Germany) after 30 min. The inhibition of luminescence was calculated relative to negative controls and the limit of detection (three times the standard deviation of the % inhibition values of the negative controls) was generally <10% inhibition. Each sample was analysed on two separate occasions and a reference compound (pentachlorophenol), negative control and inter-assay sample were included on each plate for quality control. The % inhibition was plotted against log concentration (M) and the concentration causing 50% decrease in luminescence (IC_{50}) for each household product, active ingredient and artificial mixture was calculated from the log-logistic concentration-effect curve using Equation 1 in GraphPad Prism (GraphPad Software, U.S.A.).

$$y = \frac{100}{1+10^{[(logIC_{50}-x)\times slope]}} \quad (1)$$

The algal IPAM bioassay methods (using the green algae *Pseudokirchneriella subcapitata*) have been previously described by Escher et al. (2008). Briefly, the household products, individual active ingredients and synthetic mixtures were serially diluted in Talaquil test media in a 96 well plate and then diluted 1:1 with 150 µL of algae that had an optical density (OD_{685}) of 0.1. PSII quantum yield (Y(II)) was calculated using Equation 2 by measuring the momentary fluorescence yield (F) and maximum fluorescence yield (Fm') at 0, 2 and 24 h using Imaging Pulse Amplitude Modulation (IPAM) fluorometry (Walz GmbH, Germany). The following measurement settings were used: pulse modulated measuring light intensity = 10, measuring light frequency = 8, gain = 3, actinic light = 0.

$$Y(II) = \frac{(\mathrm{Fm'}-\mathrm{F})}{\mathrm{Fm'}} \quad (2)$$

PSII inhibition at 2 and 24 h was calculated using the yield of sample ($Y(II)_{sample}$) and the yield of the control ($Y(II)_{control}$) using Equation 3. Reduced photosynthesis yield at 2 h indicates that the sample has a specific effect on the algae, namely PSII inhibition, while the 24 h reading indicates delayed phytotoxicity and includes both specific and non-specific effects (Escher et al. 2008).

$$PSII\ Inhibition\ (\%) = \left(1 - \frac{Y(II)_{sample}}{Y(II)_{control}}\right) \cdot 100\% \quad (3)$$

The herbicide diuron was used as the positive control and methanol was used as the solvent control. The methanol concentration in the assay did not exceed 1.7%, with no effect on PSII inhibition observed in the solvent control (Figure S1). All samples were run independently two to three times. The concentration causing 50% effect (EC_{50}) was derived from the log-logistic concentration-effect curve using Equation 1 in GraphPad Prism (GraphPad Software, U.S.A.) The minimum was fitted to 0% and the maximum was fitted to 100%, while the slope and EC_{50} were adjustable parameters.

Mixture toxicity modelling

To predict the toxicity of the pesticide formulations, the mixture toxicity models of concentration addition (CA) (Loewe and Muischnek, 1926) and independent action (IA) (Bliss, 1939) were applied in cases where EC_{50} values could be calculated for active ingredients and the synthetic mixture. The CA predicted EC_{50} value ($EC_{50,CA}$) was determined using Equation 4 where $EC_{50,i}$ is the EC_{50} value of the individual chemical *i* in the mixture and P_i is the fraction of individual chemical in the mixture.

$$EC_{50,CA} = \frac{1}{\sum_{i=1}^{n} \frac{P_i}{EC_{50,i}}} \quad (4)$$

The effect based on IA predictions (E_{IA}) was determined using Equation 5, where E_i is the effect of the individual chemical *i* in the mixture.

$$E_{IA} = 1 - \prod_{i=1}^{n} (1-E_i) \quad (5)$$

Results and Discussion

Bacterial toxicity

Concentration-effect curves for the commercial formulations and artificial mixtures, as well as CA and IA predictions (where possible) are shown in Figure 1. The concentration-effect curves (Figure S2) and EC_{50} values (Table 2) for the individual active ingredients, artificial mixtures and commercial formulations are also provided. The herbicide containing MCPA and dicamba (CO_H2) was the most toxic of the household products to bacterial luminescence ($IC_{50} = 4.60 \times 10^{-6}$ M), more than 600 times more potent than the least toxic product, the herbicide CO_H1a ($IC_{50} = 3.04 \times 10^{-3}$ M). Interestingly, the household herbicide CO_H1b ($IC_{50} = 5.82 \times 10^{-5}$ M) was > 50 times more toxic than CO_H1a ($IC_{50} = 3.04 \times 10^{-3}$ M), despite having exactly the same ratio of active ingredients. This further highlights that undisclosed additives, even those deemed safe individually, may not be benign as components of commercial pesticide formulations, and that the specific composition of the mixture is an important determinant of toxicity.

In almost all cases where it was possible to directly compare, the household products were more toxic than any of the corresponding artificial mixtures. This clearly indicates that additives in the household pesticides are either increasing the toxicity of the active ingredients or having a toxic effect on bacteria themselves. Specifically, the commercial insecticides CO_I1 and CO_I2 were ~50 and ~20 times more toxic to bacteria than their corresponding artificial mixtures, respectively. This is consistent with previous findings that commercial formulations of maize herbicides were more toxic to bioluminescent bacteria (*Vibrio fisheri*) than the pure compounds (Joly et al., 2013). Similarly, although a dose-response curve could not be generated for the artificial mixture AI_H1, the IC_{50} value for the

corresponding commercial formulation CO_H1b was similar to highest concentration of the artificial mixture tested, indicating likely higher toxicity of the commercial herbicide.

Interestingly, the commercial herbicide CO_H2 showed almost identical toxicity to its corresponding artificial mixture (AI_H2). Despite the good agreement in potency between the commercial product and the artificial mixture, it is likely that additives are included in this commercial product, particularly those that increase the solubility of these compounds, since the reported concentrations of MCPA (150 g/L) and dicamba (25 g/L) far exceed the water solubility of these compounds (0.63 and 8.3 g/L, respectively). In this case, however, it appears that these additives have little or no toxicity to bacteria.

Mixture toxicity modelling was only possible for herbicide H2, for which the IC_{50} values showed good agreement with that of the associated artificial mixture, and both the CA and IA predicted similar IC_{50} values (Figure 1B, Table 2). Mixture modelling was not possible for bacterial results with the other commercial products, mainly due to the low solubility of the active ingredients in methanol limiting the maximum concentrations that could be tested and hence, the number of EC_{50} values that could be attained.

Algal toxicity

The 24 h IPAM algal bioassay concentration-effect curves for the household products and artificial mixtures, as well as CA and IA predictions (where possible), are shown in Figure 2. The 24 h EC_{50} values for the individual active ingredients, artificial mixtures and commercial formulations are provided in Table 3. Both 2 h and 24 h concentration effect curves for the active ingredients, artificial mixtures and commercial formulations are provided in the Supporting Information (Figures S3 and S4).

Interestingly, the commercial insecticide CO_I1 was the most potent commercial product in the algae IPAM bioassay, despite being marketed as an insecticide rather than a herbicide.

The commercial herbicides CO_H1a and CO_H1b were also highly potent in the algae IPAM bioassay, which was more expected due to the presence of simazine (a known PSII inhibitor; Tang and Escher, 2014) in these formulations. The herbicide CO_H2 was the least potent PSII inhibitor (>450 less toxic than CO_I1), which was not unexpected as the two active ingredients (MCPA and dicamba) in this commercial formulation are synthetic auxins, which interfere with plant growth hormones rather than photosynthesis.

The active ingredients alone followed more expected patterns of phytotoxicity, with the herbicides more potent inhibitors of photosynthesis that the insecticides. Complex mixtures are recognised to often elicit unexpected or poorly understood effects (Mumtaz et al., 2010), and the present results therefore highlight the value of *in vitro* bioassays for broad screening of toxic effects related to complex chemical mixtures in commercial pesticide formulations.

The commercial herbicides CO_H1a and CO_H1b both had a specific effect on algae (similar 2h and 24 h EC_{50} values), as did their major active ingredient, simazine. However, these commercial simazine-based herbicides were over one order of magnitude less toxic than the corresponding artificial herbicide mixture AI_H1. These observed differences may have been due to the presence of additional additives in the formulation or a difference in the purity and/or potency of analytical grade simazine compared to industrial grade simazine.

In contrast, many of the other commercial formulations, artificial mixtures and individual compounds tested here showed much more effect after 24 h (Figures S2 and S3), suggesting that they mainly have a non-specific effect on algae (Escher et al. 2008). The herbicide CO_H2 had an effect on algae at high concentrations, but the corresponding artificial mixture (AI_H2) and its individual chemicals (MCPA and dicamba, both of synthetic auxins) did not inhibit PSII inhibition up to the highest tested concentration. The household insecticide CO_I1 was around 2.5 times more toxic than the corresponding artificial mixture AI_I1, and

CO_I2 was two times more toxic than the only listed active ingredient diazinon (AI_I2). As with bacteria toxicity, the lack of agreement between the commercial formulations and artificial mixtures clearly indicates the hidden effects of additives on algae.

Mixture toxicity modelling was only possible for the H1 and A1 mixtures in the algal bioassay, with both the CA and IA predicting EC_{50} values closer to the EC_{50} values of the artificial mixtures than the EC_{50} values of the commercial formulations (Table 3). The IA model was a slightly better fit than the CA model for H1, which was expected given the components of H1 have different modes of action (Belden et al. 2007), including photosynthesis inhibition (simazine) and carotenoid synthesis inhibition (amitrol). Both CA and IA slightly underestimated the toxicity of the insecticide artificial mixture AI_I1. This was not unexpected, due to the presence of piperonyl butoxide, which is a P450 inhibitor with well-known synergistic effects with pyrethroids (Kakko etl al. 2000). In all cases there was little difference between the CA and IA predictions. This fits with previous findings by Escher et al. (2017) using the non-specific Microtox assay, with differences between CA and IA predictions often not distinguishable for mixtures with few components.

Conclusions

The increased toxicity of household pesticides compared to artificial mixtures of the active ingredients, attributed here to the presence of additives in commercial formulations, are not particularly surprising. Indeed, increasing toxicity of the active ingredient is one of the reasons for including additives in pesticide formulations in the first place (Castro et al., 2014). This increased toxicity, however, has important ramifications in the context of toxicity to non-target biological processes. Due to their general use in residential areas, including application to impermeable surfaces like driveways and footpaths, the constituents in these household products are likely to end up in waterways via stormwater runoff following rainfall events. Indeed, chemicals such as simazine are regularly reported in stormwater (Bailey et al.,

2000; Rippy et al., 2017), and it could therefore be expected that the additives in simazine-based (and other) commercial pesticides would also be present in aquatic environments. This research therefore highlights the need to identify and report the additives in these products and calls for a structured approach to assessing the toxic effects of specific combinations of additives and active ingredients on aquatic organisms. Regulators and customers should require more clarity from the pesticide industry about the nature and concentrations of not only the active ingredients, but also additives used in commercial formulations.

Figures and Tables

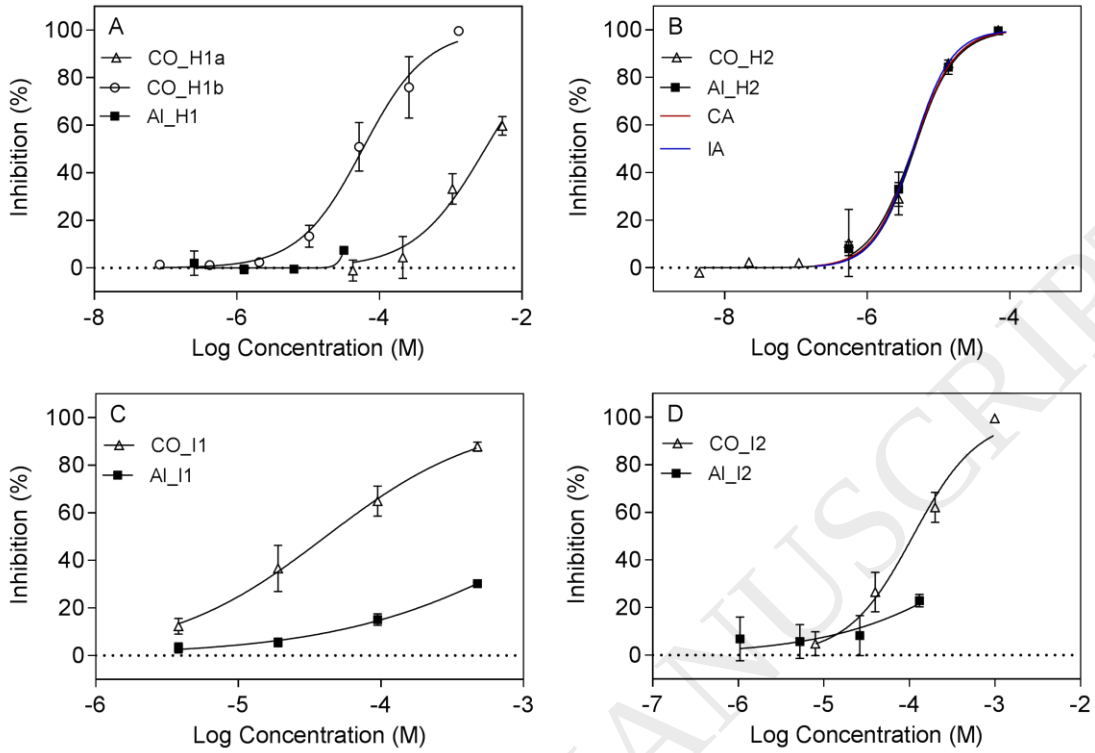

Figure 1. BLT-Screen concentration-effect curves for commercial products: A) herbicide 1 (CO_H1a and CO_H1b), B) herbicide 2 (CO_H2), C) insecticide1 (CO_I1) and D) insecticide 2 (CO_I2), and the corresponding artificial mixtures of their active ingredients (AI_H1, AI_H2, AI_I1 and AI_I2, respectively) tested in this study. Concentration addition (CA) and independent action (IA) predictions shown for B) only, as the other artificial mixtures were not sufficiently toxic to apply those models.

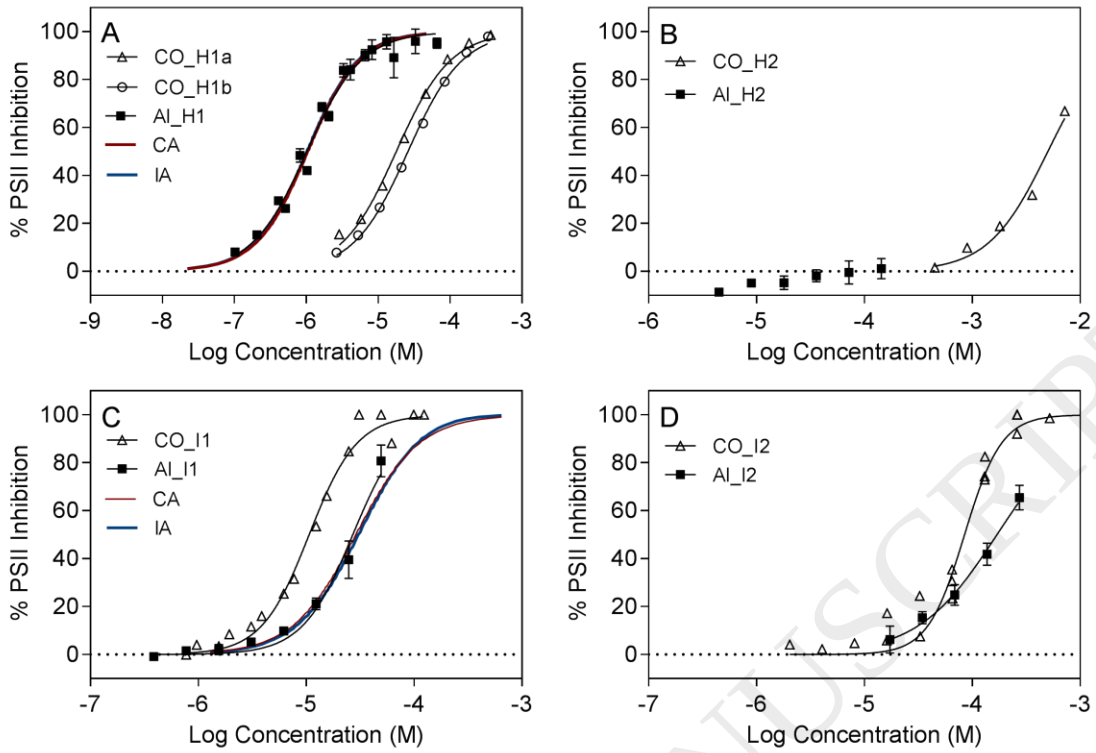

Figure 2. The 24 h algal phytotoxicity concentration-effect curves for commercial products: A) herbicide 1 (CO_H1a and CO_H1b), B) herbicide 2 (CO_H2), C) insecticide1 (CO_I1) and D) insecticide 2 (CO_I2), and the corresponding artificial mixtures of their active ingredients (AI_H1, AI_H2, AI_I1 and AI_I2, respectively) tested in this study. Concentration addition (CA) and independent action (IA) predictions shown for A) and C) only, as the artificial mixture of H2 (B) was not sufficiently toxic, and the insecticide I2 (D) only contained one active ingredient (diazinon).

Table 1. Active ingredients listed in household pest control products

Household pesticide formulations			**Corresponding active-ingredients-only mixtures**	
ID	**Class**	**Listed active ingredients and concentration**	**ID**	**Active ingredient and concentration**
CO_H1a	Herbicide	36.9 g/L simazine, 20.5 g/L amitrol, 18 g/L ammonium thiocyanate (ratio 2.05:1.14:1)	AI_H1	220 mg/L simazine, 122 mg/L amitrol, 107 mg/L ammonium thiocyanate (ratio 2.05:1.14:1)
CO_H1b	Herbicide	9 g/L simazine, 5 g/L amitrol, 4.4 g/L ammonium thiocyanate (ratio 2.05:1.14:1)		
CO_H2	Herbicide	150 g/L MCPA, 25 g/L dicamba (ratio 6:1)	AI_H2	1500 mg/L MCPA, 250 mg/L dicamba (ratio 6:1)
CO_I1	Insecticide	4 g/L pyrethrum extract, 16 g/L piperonyl butoxide (ratio 1:4)	AI_I1	200 mg/L pyrethrum extract, 800 mg/L piperonyl butoxide (ratio 1:4)
CO_I2	Insecticide	38 g/L diazinon	AI_I2	5 g/L diazinon

Table 2. Summary of IC_{50} data (M) for household products, artificial mixtures and individual active ingredients analysed in the BLT-Screen, including concentration addition (CA) and independent action (IA) predictions. 95% confidence intervals provided in brackets.

EC_{50} (M)	Individual active ingredients			Household products		Artificial Mixture	CA	IA
H1	*Simazine* $>1.0 \times 10^{-5}$	*Amitrol* $>1.14 \times 10^{-2}$	*Ammonium thiocyanate* 3.02×10^{-3} (2.55 to 3.59)	*CO_H1a* 3.04×10^{-3} (1.97 to 4.67)	*CO_H1b* 5.82×10^{-5} (4.35 to 7.77)	*AI_H1* $>3.2 \times 10^{-5}$	-	-
H2	*MCPA* 4.20×10^{-6} (3.21 to 5.49)		*Dicamba* 7.33×10^{-6} (5.76 to 9.33)	*CO_H2* 4.60×10^{-6} (3.68 to 5.76)		*AI_H2* 4.36×10^{-6} (3.63 to 5.23)	4.45×10^{-6} (3.65 to 5.43)	4.45×10^{-6} (3.69 to 5.36)
I1	*Pyrethrum extract* 1.17×10^{-3} (0.90 to 1.52)		*Piperonyl butoxide* 2.92×10^{-3} (1.03 to 8.28)	*CO_I1* 4.10×10^{-5} (2.99 to 5.61)		*AI_I1* [a]2.01×10^{-3} (1.29 to 3.14)	-	-
I2			*Diazinon* 2.0×10^{-3} (0.004 to 91.3)	*CO_I2* 1.10×10^{-4} (0.78 to 1.55)		*AI_I2 (diazinon)* 2.0×10^{-3} (0.004 to 91.3)	-	-

[a] extrapolated from concentration effect curve ending at 30% inhibition

Table 3. Summary of EC_{50} values (M) for household products, artificial mixtures and individual active ingredients analysed in the algae PSII inhibition assay, including concentration addition (CA) and independent action (IA) predictions. 95% confidence intervals provided in brackets.

EC_{50} (M)	**Active ingredients**			**Household products**		**Artificial Mixture**	**CA**	**IA**
H1	*Simazine* 2.91×10^{-7} (2.73 to 3.10)	*Amitrol* 5.09×10^{-4} (4.29 to 6.05)	*Ammonium thiocyanate* $>4.38\times10^{-4}$	*CO_H1a* 1.79×10^{-5} (1.62 to 1.97)	*CO_H1b* 2.60×10^{-5} (2.47 to 3.73)	*AI_H1* 1.02×10^{-6} (0.95 to 1.10)	1.05×10^{-6} (0.99 to 1.12)	1.02×10^{-6} (0.96 to 1.09)
H2	*MCPA* $>1.66\times10^{-4}$		*Dicamba* $>7.54\times10^{-5}$	*CO_H2* 5.01×10^{-3} (4.50 to 5.56)		*AI_H2* $>1.43\times10^{-4}$	-	-
I1	*Pyrethrum extract* 6.15×10^{-5} (4.78 to 7.92)		*Piperonyl butoxide* 2.54×10^{-5} (2.33 to 2.77)	*CO_I1* 1.07×10^{-5} (0.99 to 1.14)		*AI_I1* 2.71×10^{-5} (2.50 to 2.93)	2.89×10^{-5} (2.67 to 3.13)	3.05×10^{-5} (2.82 to 3.30)
I2	*Diazinon* 1.69×10^{-4} (1.52 to 1.88)			*CO_I2* 8.47×10^{-5} (7.80 to 9.20)		*AI_I2 (diazinon)* 1.69×10^{-4} (1.52 to 1.88)	-	-

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