Environment International 37 (2011) 663–670

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Modelling the joint effects of a metal and a pesticide on reproduction and toxicokinetics in Lumbricid earthworms

L.J. Lister [a], C. Svendsen [b], J. Wright [a], H.L. Hooper [b], D.J. Spurgeon [b,*]

[a] Centre for Ecology and Hydrology, Monks Wood, Abbots Ripton, Huntingdon, Cambridgeshire PE28 2LS, UK
[b] Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire OX10 8BB, UK

ARTICLE INFO

Article history:
Received 4 October 2010
Accepted 13 January 2011
Available online 16 February 2011

Keywords:
Concentration addition
Independent action
Toxicokinetics
Bioconcentration factor
Cytochrome p450
Toxicodynamics

ABSTRACT

It is important to understand the aetiology of interactive mixtures effects (i.e. synergism and antagonism) if results from known cases are to be extrapolated to untested combinations. The key role of toxicokinetics in determining internal concentrations at target sites means that understanding chemical uptake in mixtures is an essential requirement for mechanistic understanding of interactions. In this paper, a combined approach using mixture toxicity testing, toxicokinetic studies and modelling has been used to address the link between joint toxicity and internal concentration. The study is conducted in Lumbricid earthworms with a binary mixture of a metal (nickel) and an organophosphate insecticide (chlorpyrifos) not *a priori* expected to show interactive toxicity.

As expected from their dissimilar modes of action and detoxification, exposure to combinations of nickel and chlorpyrifos resulted in additive toxicity. Measurement of internal concentrations indicated that both chemicals were rapidly accumulated (within 3 days) to equilibrium. When exposed as a mixture, Ni uptake followed the same pattern as found for the single chemical. This was not the case for chlorpyrifos which showed a faster rate of uptake and elimination and a slightly higher equilibrium concentration in a mixture. That the difference in chlorpyrifos kinetics in the mixture did not result in interactive toxicity highlights the need to assess chemical toxicodynamics as well as toxicokinetics. Measurement of chlorpyrifos-oxon identified the presence of this toxic form but implementation of more complex approaches encompassing toxicogenomics and epigenetics are ultimately needed to resolve the toxicokinetic to toxicodynamic link for these chemicals.

1. Introduction

Chemical risk assessments are conducted for individual chemicals according to standardised frameworks such as the Registration, Evaluation, Authorisation and Restriction of CHemicals legislation of the European Union. In the environment, however, organisms are frequently exposed to a mixture of chemicals of both related and distinct classes. While the effects of multiple chemicals can often be additive, in accord with predictions of the concentration addition (CA) and/or independent action (IA) models (Altenburger et al., 2000; Backhaus et al., 2004; Faust et al., 2003), the potential for interactive toxicity is evident (Belden et al., 2007; Cedergreen et al., 2008; Jonker et al., 2005). The issue of interactive toxicity in mixtures is often highlighted in ecological risk assessments, yet the toxicokinetic and toxicodynamic mechanisms responsible for such interactions are rarely investigated, meaning that the cause remains obscure and so difficult to extrapolate between causes.

To investigate the causes of mixture interactions, studies of the main processes that govern the nature and extent of toxicity, namely bioavailability, toxicokinetics and toxicodynamics are needed (Spurgeon et al., 2010). Previous studies have identified changes in chemical toxicokinetics (rates of chemical adsorption, distribution, metabolism and excretion) as one of the main causes of interactions affecting joint toxicity (Cedergreen et al., 2008; Walker, 2008). Because of their key role in toxicokinetics, binding and trafficking pathways, influx/efflux pumps and metabolising enzymes (metal binding proteins, ABC-transporters, cytochrome p450s and glutathione-s-transferase) can be important mediators of mixture interactions through effects on the rates of toxicokinetic processes (Dorne et al., 2007; Walker, 2008). When interactive effects on these processes cause whole body and especially target tissue concentrations for one or both chemicals to depart from the single chemical case, then greater (synergistic) or reduced (antagonistic) toxicity can result.

The key role of toxicokinetic in governing concentrations at target sites means that the understanding of mixture effects is improved when patterns of uptake for chemicals within a mixture are known. Such time dependent accumulation patterns in a mixture can be

Abbreviations: GC–MS, Gas chromatography mass spectrometry; CA, Concentration addition; IA, Independent action; CPF, Chlorpyrifos; CPF-oxon, Chlorpyrifos oxon; TU, Toxic unit; BCF, Bioconcentration factor.

* Corresponding author. Tel.: +44 1491 692208; fax: +44 1491 692424.
E-mail address: dasp@ceh.ac.uk (D.J. Spurgeon).

0160-4120/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envint.2011.01.006

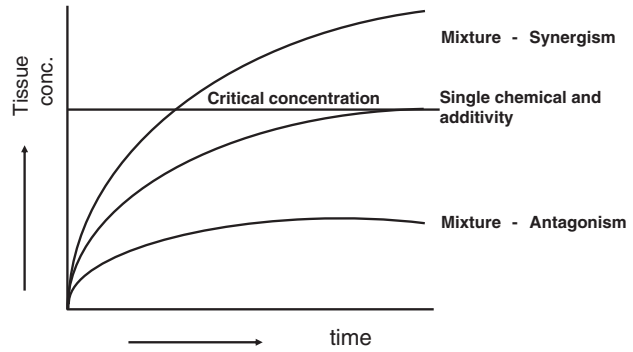

Fig. 1. Schematic representation of the effects of interaction on accumulation of a chemical: in this example tissue concentrations approach an effect threshold when presented as a single chemical and in mixtures where effects are additive, while synergism results in concentration above the effect threshold and antagonism means that the effect threshold is not reached.

compared to the single chemical case; with greater uptake characterising synergism, reduced uptake antagonism and similar uptake additivity (Fig. 1). In this paper we used a combined approach of mixture effect modelling and analysis of chemical toxicokinetics to examine the effects of a binary mixture to Lumbricid earthworms following the scheme set out in Fig. 2. The binary combination studied comprised the metal nickel (Ni) and the organophosphate insecticide chlorpyrifos (CPF). These two chemicals were selected because they have different modes of action and detoxification mechanisms. Ni is non-specific toxicant with effects on redox cycling, DNA integrity and metalloprotein integrity that is detoxified by metal chaperones (Sakar, 1999; Seo et al., 2005); CPF in contrast has a putative specific mode of action through acetylcholinesterase inhibition at the synaptic junction, with detoxification via cytochrome p450 biotransformation (Walker, 2008). Since the modes of action and detoxification for each of the two selected chemicals act separately, *a priori* interaction was not expected, thus, potentially provided the opportunity to study mixture toxicokinetics under the null case of additivity.

2. Material and methods

2.1. Mixture toxicity study

Range finder experiments for Ni and CPF were conducted with *Lumbricus rubellus* (Hoffmeister 1843) under the test conditions used for the mixture test (see below). The 28 day EC_{50}s for cocoon production of 163 mg Ni/kg soil and 32.9 mg CPF/kg soil calculated from these tests were used to design the mixture experiment.

In the mixture test, *L. rubellus* were exposed to a series of toxic unit (TU) levels for each of the two single chemicals and also for three different mixture ratios (3:1, 1:1 and 1:3), representing equitoxicity and the case where each chemical dominated the mixture. The maximum exposure level was set at 6 TUs for each single chemical and ratio. This was then sequentially divided by a log factor of 1.54 to generate a concentration series of 0.19, 0.29, 0.45, 0.7, 1.07, 1.64, 2.53, 3.9 and 6 TUs. To allow mixture effects to be statistically compared to CA ad IA model predictions it is more important to have a large number of doses in the experiment than replication of only a few doses (Jonker et al., 2005). Therefore no replicates of exposed treatments were used except for controls which were replicated eight times to provide a measure of variation in the test.

Each experimental unit consisted of a 2 l plastic container with a perforated lid containing 1.4 kg (dry wt.) of a commercially available loam (Broughton Loams, Kettering, UK) amended with 3% composted bark (LBS Horticultural, Colne, UK) as additional organic matter (Spurgeon et al., 2003). Soils were first spiked with a stock solution of nickel chloride (hexa-hydrate) (SigmaAldrich) and then made up to 50% of the water holding capacity by addition of 255 ml of deionised water. Soils were left for 13 days to allow initial equilibration of Ni

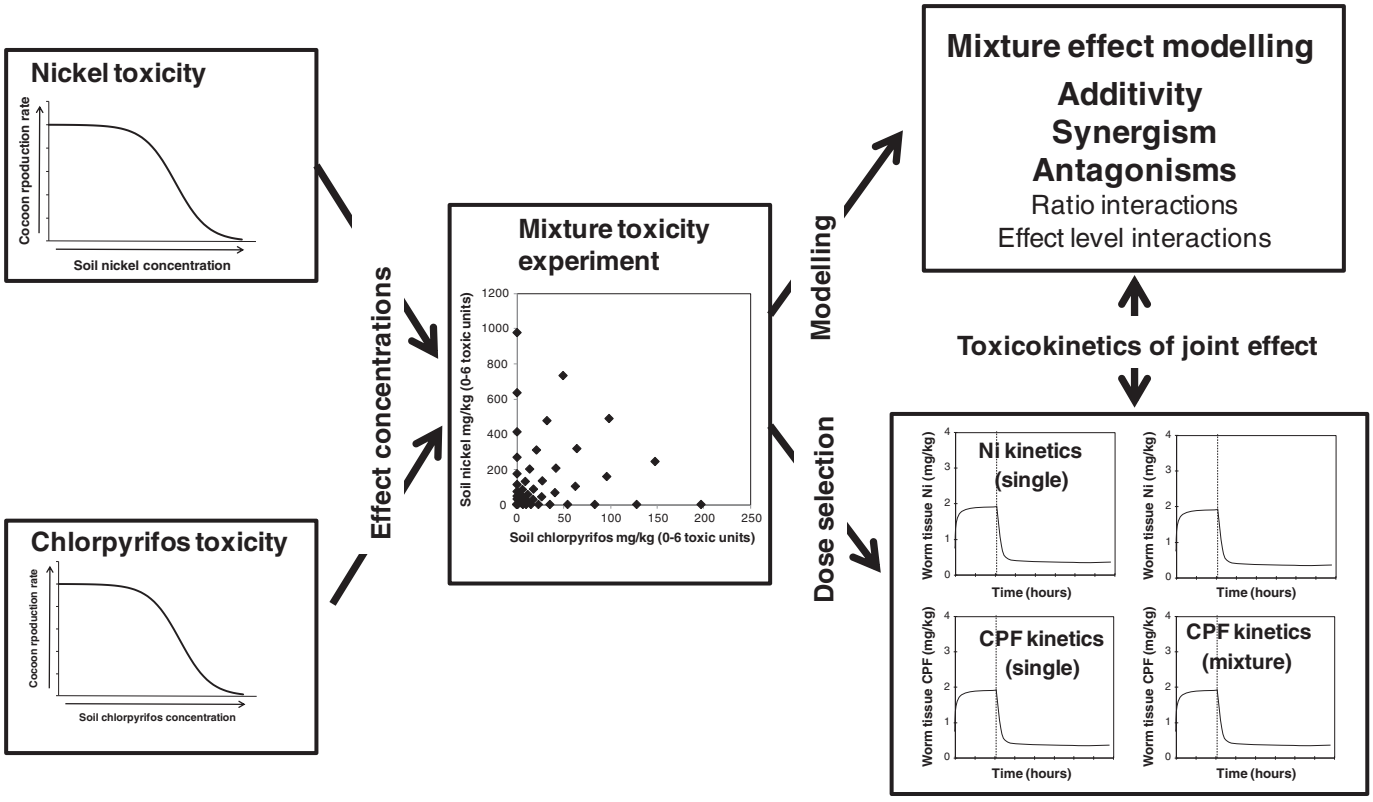

Fig. 2. Schematic of the experimental design and approaches used for utilisation and collection of mixture toxicity and toxicokinetic data.